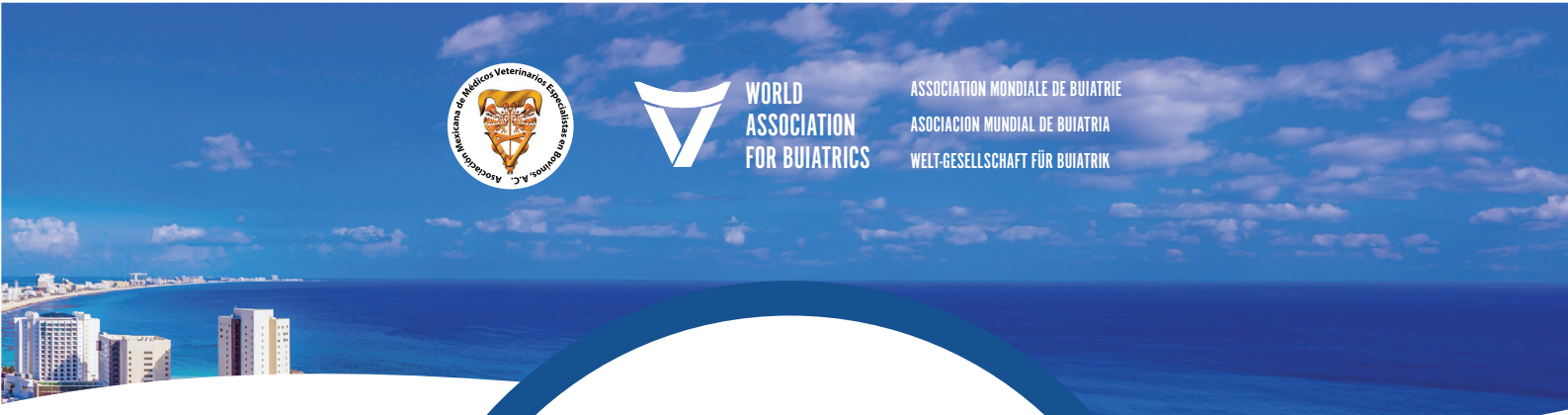

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32 WBC

Proceedings

Keynote Lectures, Oral and Poster Presentations

The 32nd World Buiatrics Congress
May 20 to 24, 2024
Cancun, Quintana Roo, Mexico

32nd World Buiatrics Congress

May 20 to 24th, 2024
Cancun, Quintana Roo, Mexico

Organizers:

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	Year	Venue		Year	Venue
1	1960	Hannover (Germany)	17	1992	St Paul (USA)
2	1962	Vienna (Austria)	18	1994	Bologna (Italy)
3	1964	Copenhagen (Denmark)	19	1996	Edinburgh (Scotland)
4	1966	Zurich (Switzerland)	20	1998	Sydney (Australia)
5	1968	Opatija (Yugoslavia)	21	2000	Punta del Este (Uruguay)
6	1970	London, UK	22	2002	Hannover (Germany)
7	1972	Philadelphia (USA)	23	2004	Quebec (Canada)
8	1974	Milan (Italy)	24	2006	Nice (France)
9	1976	Nice (France)	25	2008	Budapest (Hungary)
10	**1978**	**Mexico City (Mexico)**	26	2010	Santiago (Chile)
11	1980	Tel Aviv (Israel)	27	2012	Lisbon (Portugal)
12	1982	Amsterdam (The Netherlands)	28	2014	Cairns (Australia)
13	1984	Durban (South Africa)	29	2016	Dublin (Ireland)
14	1986	Dublin (Ireland)	30	2018	Sapporo (Japan)
15	1988	Palma de Mallorca (Spain)	31	2022	Madrid (Spain)
16	1990	San Salvador da Bahia (Brazil)	**32**	**2024**	**Cancun, Quintana Roo (Mexico)**

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PREFACE

The Mexican Buiatrics Association is proud to have organized the 32nd World Buiatrics Congress 2024 in Cancún, Mexico, an unprecedent event that brought together experts, leaders and professionals from around the world, who discussed the development and advances of the ruminant livestock industry.

Mexico has an important and diversified livestock, that plays a crucial role in the country's economy. With a historical tradition in the production of meat and milk. Mexico is one of the largest producers and exporters of beef and one of the top 20 milk producers worldwide, employing thousands of people committed to quality and sustainability.

The objectives of the Mexican Association of bovine practitioners, comprise the continuous and permanent education of veterinary doctors specialized in medicine and animal science of cattle, goats and sheep. Since its foundation in 1976 it has organized scientific conferences, and about 45 national buiatrics congresses, including two Latin American congresses. In the year of 1978, Mexico successfully organized the X World Buiatrics Congress in Mexico City, always taking into account the professional needs of the veterinary practitioners dedicated to this livestock activity.

This year 2024, we have the opportunity to host this important scientific event, allowing participants to share knowledge, solutions and innovative practices to improve ruminant livestock and productivity. During this congress we had the opportunity to share ideas and acknowledge perspectives on current and future challenges in the industry, to work together in the search for sustainable and effective solutions.

Also in this congress, with the participation of the pharmaceutical industry we had the opportunity to talk with experts and to know the development of new products and its applications in the control of diseases, parasites, vaccines, colostrum substitutes, etc.

Organizing committee of the 32nd Word Buiatrics Congress, Mexico, Cancún, Quintana Roo, May 20 to 24, 2024.

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Infectious diseases

Expectation and directions for the prudent use of antibiotics in bovine mastitis

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The development of antimicrobial resistance (AMR) currently represents one of the most important public health challenges. Any use of antibiotics carries the risk of enhancing the development of AMR. A recent report on antimicrobial use in the EU from the European Medicines Agency (EMA), European Food Safety Authority (EFSA), European Centre for Disease Control (ECDC) and Organisation for Economic Cooperation and Development (OECD) found there was “more progress in agriculture than in the human sector” and since 2016, “average consumption of antibiotics in humans is now higher than in food-producing animals”. Any way on the base of this interesting results both veterinary and human medicine have to address the problem of AMR and promote a more prudent use of antibiotics.
In dairy cows, bovine mastitis still represents one of the most common infectious diseases and treatment of mastitis remains one of the most discuss points and reason for antibiotic treatment in dairy cows. But what we need to consider, on based on USA national sales data, that intramammary treatments comprise < 1% of overall antimicrobial use for food-producing animals, but a large proportion of that usage specially in USA is a third-generation cephalosporin, which is classified as a highest-priority, critically important antimicrobial. Opportunities exist to improve the use of antimicrobials in dairy practice and to promote prudent use of antibiotics in mastitis area it is important to understand, collect and record the current treatment strategies and approaches. Also for this, different mastitis experts from Spain, The Netherlands, Estonia, Ireland, Poland, Finland, Germany, and Italy focused on protocols and they have described how clinical mastitis in lactation was usually treated in their countries.
. On many farms, almost all mastitis is treated using antibiotics but not all cases of nonsevere CM will benefit from antimicrobial therapy and protocols used to treat these cases should include alternatives strategies for managing those cases. The purpose of this project was to review use of antimicrobials for treatment of nonsevere CM to help veterinarians develop protocols that ensure responsible usage. In the United States, there are 7 approved IMMs; no systemically administered antibiotics are approved for treatment of mastitis and IMM antibiotics should be used as the first choice for treatment of nonsevere CM. Approved IMM products have pharmacologic characteristics that ensure a sufficient concentration of the drug (or active metabolite) will be present in the udder during the approved dosing interval to kill or restrict growth of the organisms listed on the product label. Guidelines for appropriate use of antibiotics

have been developed and should be applied to mastitis treatments. The most significant guidelines are that antibiotic usage should involve veterinary guidance and extralabel use should be avoided when on-label use is a possibility. Veterinarians and producers in the world should be aware of label indications and claims of efficacy and recognize that extralabel treatments occur when systemic antibiotics are administered or when the dosing regimen of intramammary products is altered from that described on the label. Deviations from label guidelines used to be common for mastitis treatment and may be justifiable for some drugs but now need, it's could be possible avoid, and to must be done always under veterinary supervision. Appropriate usage guidelines also specify that antibiotics should only be used when there is a reasonable likelihood that a bacterial infection that is sensitive to the proposed antibiotic is present. On the base of these data we need to remember that 20% to 40% of CM cases are culture negative, this criterion is often not achieved; alternative ways to manage these case should be considered. Antibiotics should not be used for cows that are unlikely to benefit and selective treatment based on on-farm or veterinary clinic laboratories is advised. Practitioners should also ensure that antimicrobials are not given to cows affected with nonsevere CM caused by a refractory pathogen, such as Mycoplasma bovis, S aureus, Prototheca, and Serratia. Depending on intrinsic bacterial susceptibility, antibiotics are classified as either narrow or broad spectrum. Narrow-spectrum drugs are usually active against either Gram-positive or Gram-negative bacteria, whereas broad-spectrum drugs have activity against both types of organisms. The World Health Organization has classified antibiotics based on their importance for treating human illnesses, and responsible usage guidelines suggest that narrow spectrum antibiotics that are less critical for treating human illnesses should be used as a first choice. Most IMM products available in Europe and the United States are not high-priority drugs for treatment of human illnesses and only ceftiofur (a third-generation cephalosporin) intramammary in USA is listed as both high priority and critically important for human use. Most approved IMM products are considered narrow spectrum, and the use of the broader-spectrum IMM drugs should be reserved for cases that will benefit.

Responsible usage guidelines propose that antibiotics should be used for as short a duration as possible. The appropriate duration of antibiotic treatment of CM is not well defined and varies depending on the etiology. There is limited evidence that extended-duration antibiotic therapy increases the bacterial cure of invasive pathogens (such as S aureus and some environmental streptococci species). However, no research has indicated that extended-duration therapy improves clinical outcomes of mastitis caused by noninvasive pathogens (such as NAS or most E coli). It is important to note that when extended IMM therapy is considered, veterinarians need to assess the ability of farm workers to perform aseptic infusions, as extended IMM treatment is associated with an increased risk of infection from opportunistic pathogens.
The decision to use an antibiotic for the treatment of nonsevere CM should be based on a reasonable expectation that an active bacterial infection is occurring in a cow who has a reasonable probability of responding to treatment using an antimicrobial with an appropriate spectrum of activity and that the use of the antibiotic will result in clinical outcomes that exceed expectations if antimicrobials are not administered.

Another important point for the reduction of antibiotics is selective dry cow therapy. Treatment of all dairy cows at drying-off has been practiced for decades; such treatment

serves a twofold purpose of eliminating a large number of subclinical infections and preventing new infections in the early dry period. Blanket dry cow therapy still provides one of the corner stones in mastitis control in many countries. The practice of blanket dry cow therapy has recently been questioned, since bulk milk tank somatic cell counts have markedly decreased and the principal causative agents of mastitis has changed from contagious to environmental. Selective dry cow therapy (i.e. identification and treatment of cows with intramammary infection) is an increasingly attractive method to decrease routine antimicrobial use in dairy cattle. Refinement of currently available screening tests for intramammary infection (such as somatic cell count, California Mastitis test results or electrical conductivity) that produces a test with adequate sensitivity and specificity will make selective dry cow therapy a routine recommendation for herds. It is not economical to treat cows infected by the so-called minor pathogens. Systemic administration of antimicrobials has been proposed for dry cow therapy, but no scientific evidence has been presented to support the better efficacy of this practice. An internal teat sealer for prevention of new infections shows promise as a non-antibiotic alternative for preventing new intramammary infections during the dry period. In some countries, prepartum intramammary antimicrobial therapy has been introduced as a means to control mastitis in heifers. This cannot be regarded as a prudent use of antimicrobials; furthermore, the advantages from this practice have been questioned.

Summary

Appropriate use of antimicrobials on dairy farms contributes to improving animal well-being and dairy farm sustainability but it is important for veterinarians to recognize that many cases of nonsevere CM will not benefit from antimicrobial therapy. Mastitis is caused by a diverse group of bacterial pathogens with differing distributions among farms. In intensively managed herds, many cases of CM are culture-negative when detected or are caused by pathogens with high rates of spontaneous cure. In such herds, when treatments are administered without knowledge of etiology, most antimicrobial treatments are likely to be unnecessary. There is considerable opportunity for veterinarians to improve antimicrobial usage on dairy farms by encouraging farmers to adopt culture-based treatment protocols that limit antimicrobial usage to cases that will benefit. When this option is not feasible, farmers should be encouraged to review the medical history of the cow before treatment and, when antimicrobial use is warranted, initiate therapy using a narrow-spectrum drug for a short duration.

Epidemiological characteristics of pathogens currently causing bovine mastitis on large dairy farms in China

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Abstract

Bovine mastitis is a major disease that adversely affects dairy cow health and causes serious economic losses. Selective antimicrobial treatment protocols based on appropriate diagnostic tests can effectively control bovine mastitis pathogens. However, due to substantial recent changes in management and mastitis treatment, current epidemiological characteristics and pathogens causing bovine mastitis on large dairy farms in China are unclear. Therefore, the objective was to determine microorganisms most frequently associated with mastitis in dairy cows in China, and their antibiotic sensitivity. In total, 3126 bovine mastitis milk samples, including 2618 clinical mastitis milk samples and 376 bulk tank samples were collected from 255 herds in 22 provinces of China in 2022 and 2023 and analyzed. There were 2612 pathogenic isolates, with 1851 (70.9%) categorized as environmental pathogens, 261 (10.0%) contagious pathogens, and 500 (19.1%) others. The most common pathogens included environmental pathogens NAS 23.6% (615/2612), followed by Klebsiella spp. 20.8% (544/2612), E. coli 13.4% (349/2612), and S. dysagalactiae and S. uberis 9.2% (240/2612), plus contagious pathogens Mycoplasma bovis 9.1% (114/1254), Streptococcus agalactiae 3.0% (79/2612), and Staphylococcus aureus 2.6% (68/2612). The majority of isolates were from Northern China, Northwest and Southwest China, and Eastern China. Pathogens were mainly detected in Spring, Summer and Autumn, with isolation in Summer and Autumn significantly higher than the Spring and Winter. Selective clinical mastitis treatment and alternative treatments can be practical tools to reduce antimicrobial use on dairy farms. These findings contributed to risk assessment of selective treatment and provided a baseline for establishing and evaluating control measures and designing strategies to reduce development of antimicrobial resistance. It was apparent that there were reductions in contagious pathogens.

Introduction

Bovine mastitis represents a significant challenge for the global dairy industry, leading to substantial economic losses through reduced milk production, discard of wasted milk, livestock culling, medication and labor costs(**Bhakat et al., 2020)(1)**. The condition is predominantly caused by bacterial infections including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, *Escherichia coli*, *Klebsiella spp.*, non-aureus staphylococci (NAS), *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Enterococcus spp.* (**Klaas and Zadoks, 2018**)(**2**). Accurate identification of the pathogens present within a herd is essential for appropriate prevention and control measures, and for developing treatment protocols with optimal antibiotic choices (**Botrel et al., 2010**) (**3**). The distribution of pathogens responsible for clinical mastitis varies globally, influenced by regional practices, environmental conditions, and herd management.

In Brazil, the most frequent pathogen causing mastitis in dairy cattle was *Staphylococcus* spp; it was isolated in all studies and had an average prevalence of 49%

(**Ito Eleodoro et al., 2022**) (**4**). In India, *Staphylococcus aureus* was the major mastitis pathogen, mostly causing subclinical mastitis, with *Escherichia coli* causing clinical mastitis and *Streptococcus* spp. causing subclinical and clinical mastitis (**Krishnamoorthy et al., 2021**) (**5**).

There is an urgent need to reduce mastitis pathogen prevalence by ensuring scientific farm management practices, proper feeding, and therapeutic interventions to improve animal and human health and promote profitable dairy production (**Krishnamoorthy et al., 2021**) (**5**). In America, *Mycoplasma bovis* was the most prevalent Mycoplasma species (75.1%) in mastitic milk (Gioia et al. 2021), indicating the importance of testing milk samples for mycoplasmas using diagnostic tests able to identify both genus and species (**Gioia et al., 2021**) (**6**). In America, the incidence rate of clinical mastitis was 24.4% (**Gonçalves et al., 2022**) (**7**). In Europe, the most common causes of bovine mastitis were *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli*, NAS, and other *Streptococcus* spp. (**Naranjo-Lucena and Slowey, 2023**) (**8**). In Canada, the major bacterial pathogens were *Staphylococcus aureus* (15.60%), NAS (5.04%), *Corynebacterium* spp. (2.96%), and *Escherichia coli* (2.00%) (**Acharya et al., 2021**) (**9**). Of the NAS, the major species reported were *Staphylococcus chromogenes* (69.02%), *Staphylococcus simulans* (14.45%), *Staphylococcus epidermidis* (12.99%), and *Staphylococcus hyicus* (2.13%). Isolation of most mastitis pathogens peaked in summer, except that *Staphylococcus aureus, T. pyogenes*, and *Streptococcus dysgalactiae* peaked in spring (**Acharya et al., 2021**) (**9**). In China, the incidence of clinical mastitis (CM) was 3.3 cases per 100 cows per month (range = 1.7 to 8.1), with the majority of isolates being environmental pathogens, followed by contagious pathogens (**Gao et al., 2017**) (**10**). Based on epidemiological data, environmental pathogens are the leading cause of mastitis globally, as predicted (**Ruegg, 2017**) (**11**).

Various factors, such as season, climate, herd size, and bedding material, influence the spread of pathogens causing bovine mastitis (**Song et al., 2020**) (**12**). Certain pathogens are more common in warm, humid climates, whereas others dominate in cold, dry environments (**Ameni et al., 2022**) (**13**). Pathogens common in dry sand, straw, and dried manure bedding were reported (**Bradley et al., 2018**) (**14**). This detailed analysis of factors affecting pathogen distribution is vital for creating focused prevention and control strategies, highlighting the role of environmental management in reducing mastitis risk.

In 2017, we detailed the distribution of CM pathogens in large dairy farms across all herds. However, since then, dairy farming in China has evolved with improved management, higher milk production, adoption of recycled manure solids (RMS) as bedding, and stricter veterinary antibiotic usage, aiming to better control contagious mastitis. However, effects of these changes on CM pathogen types is unclear. Therefore, the current study aimed to elucidate the prevalence of CM pathogens in large Chinese dairy farms in 2022 to 2023 and their association with farm-level factors. These findings were compared to data from 2014 to 2016 to assess trends in pathogen prevalence. The outcomes should offer valuable insights for targeted CM prevention and control on Chinese dairy farms and guide treatments.

Materials and methods

Dairy herd information

This study involved 255 dairy farms across 22 provinces in China from 2022 to 2023. Sampled areas were divided into four regions, with 26 farms in the Northeast, 105 in Northern China, 60 in the Northwestern and Southwestern areas, and 64 in Eastern China. Farms varied in size, with 1000 to 12,000 lactating cows. These farms implemented modern dairy practices, including total mixed ration feeding systems and milking in rotary or parallel parlors.

A consistent diagnostic criterion for mastitis was used across the farms, categorized into three grades: Grade I mastitis was identified by changes in milk appearance; Grade II included milk changes plus clinical signs that included redness, heat, swelling, and pain in the udder; and Grade III involved local (udder) clinical signs, along with systemic clinical signs such as fever, lethargy and depression. Treatment protocols varied by mastitis grade, with Grade I typically receiving intramammary antibiotics, Grade II the same with possible NSAIDs, and Grade III a combination of intramammary infusion antibiotics, NSAIDs, systemic antibiotic injections, and supportive care (IV fluids and calcium supplements).

All participating farms employed free-stall housing, using a variety of bedding materials including recycled manure solids (RMS), rice hulls and dry sand, with some using a blend of rice hulls and RMS. A complete dry cow therapy protocol was used, involving infusion of a long-acting antibiotic preparation into each teat at drying off. Approximately half of the farms also used teat sealants. Although complete records of mastitis cases and treatments were kept across all farms, only approximately one-third maintained Dairy Herd Improvement records.

Aseptic sampling and recording

Herd veterinarians were responsible for aseptically collecting milk samples from quarters with clinical signs of CM before starting antibiotic treatment. Farm staff routinely identified potential CM cases during milking, which were then verified by a veterinarian. Veterinarians aimed to sample from every infected quarter, checking for abnormal milk or swelling, prior to any treatment. Although there was no set quota for CM sample submissions, larger herds were encouraged to submit more samples. Despite often receiving multiple batches of samples from herds, in most cases, only the first batch was considered for inclusion in the study.

Our laboratory provided sampling kits that included sample tubes, recording forms, labels, and instructions for aseptic milk sample collection. The sampling followed strict aseptic protocols: after discarding the first 3 to 5 milk streams, teats were disinfected and dried. Teat ends were then swabbed with 75% alcohol until completely clean and allowed to air-dry for 3-5 s. Milk samples, 5 to 10 mL each, were aseptically squirted into sterile centrifuge tubes, labeled, and transported under cold conditions to our laboratory for bacterial culture.

Key farm information, including date of sampling, herd size, and bedding material were documented, based on farm records. However, specific details such as parity, days in milk, severity of clinical signs, and treatment protocols were not routinely recorded.

Pathogen identification

Upon reaching our laboratory, all frozen milk samples were thawed at room temperature. For culture, ~10 µL aliquot of each milk sample was spread onto blood agar plates, which were incubated at 37°C for 24 h. A sample was marked as culture-positive if 1 or 2 colony forms were presented, whereas samples with 3 or more types

of colonies were considered contaminated, except in cases where *Staphylococcus aureus* or *Streptococcus agalactiae* were identified.

After initial incubation, selected colonies were subjected to Gram staining and re-cultured for secondary isolation, with concurrent microscopic examination. Gram-negative bacilli were plated onto citrate agar, MacConkey (MAC) agar, and eosin methylene blue (EMB) agar, whereas Gram-positive cocci were plated onto mannitol salt agar. These cultures were incubated at 37°C for 24 h to identify changes in media and colony appearances. A shift to yellow on mannitol salt agar signified staphylococci presence; red colonies on MAC agar or black colonies with a metallic green sheen on EMB agar suggested *Escherichia coli*; and pink colonies with a yellow center on MAC agar and blue coloration on citrate agar indicated *Klebsiella* spp. Organisms not identified by these methods were subjected to 16S rDNA sequencing for accurate species determination.

For mycoplasma detection, the culture process aligned with National Mastitis Council (NMC) guidelines (2017) (**15**), starting with the milk sample being inverted and mixed, then spread onto PPLO (pleuropneumonia-like organisms) solid medium using a sterile cotton swab. These plates were incubated at 37°C with > 95% humidity and 5% CO_2 for 5-10 d. Following incubation, samples were examined under a microscope for colony morphology indicative of mycoplasma. Positive colonies were further cultured in PPLO liquid medium under identical conditions for 72 h. A change to yellow in the medium prompted use of PCR to confirm the presence of *M. bovis*.

Statistical Analyses

Statistical analyses were conducted using SPSS Version 20.0, setting the significance threshold at $P< 0.05$ and reporting means and standard deviations. To compare region, season, farm size, and bedding type, Chi-square was utilized.

Results

Pathogens

In total, 3126 bovine mastitis milk samples from 255 herds in 22 provinces of China were collected and analyzed in 2022 and 2023 (**Table 1**). Among them, there were 2618 clinical mastitis milk samples and 376 bulk tank milk samples. Finally, 2612 pathogenic isolates were identified, which included 1851 (70.9%, 1851/2612) environmental pathogens isolates, 261 (10.0%, 261/2612) contagious pathogens, and 500 others (19.1%, 500/2612) . The most frequently isolated pathogens included NAS 23.6% (615/2612), followed by *Klebsiella* spp. 20.8% (544/2612), *E. coli* 13.4% (349/2612), *S. dysagalactiae* and *S. uberis* 9.2% (240/2612), *Mycoplasma bovis* 9.1% (114/1254), *Streptococcus agalactiae* 3.0% (79/2612), and *Staphylococcus aureus* 2.6% (68/2612).

Area

In total, 412 bovine mastitis milk samples were collected from 26 dairy farms of Northeastern China, plus 1135, 803 and 775 samples from 105 dairy farms in Northern China, 60 dairy farms in Northwest and Southwest Chian, and 64 dairy farms from Eastern China, respectively **(Table 1)**. Distribution of selected pathogens isolated from 3126 mastitis milk samples collected between 2022 and 2023 from 255 large (> 500 lactating cows) dairy herds in four regions of China are shown (**Table 2)**. *Klebsiella* spp. and non-aureus Staphylococci were common in all four regions, but *E. coli* was only common in Northern and Northwest & Southwest area. There were variations among regions in isolation of *Mycoplasma bovis*, with significantly higher isolation rates in

Northeast China (11.1%) than the North China (4.9%), West China (5.1%) and Southern China (5.1%). Other bacteria were at low levels.

Season

A total of 915 isolates were isolated from 858 bovine mastitis milk samples in 71 farms in 2022 and 2023, for an average isolation of 457.5 per year. On average there were 41 contagious isolates and 219 environmental isolates in Spring. Furthermore, during summer, there were 1169 isolates isolated from 981 milk samples on 92 farms in the last 2 years (average of 584.5 per year). On average, there were 30.5 and 269 contagious and environmental isolates, respectively. For Autumn, there were 1376 isolates isolated from 793 milk samples on 73 farms (average of 688 per year), with averages of 39.5 contagious and 225 environmental strains. Finally, 469 isolates were isolated from 455 milk samples on 39 farms on Winter (average of 234.5 per year). There were an average of 14.5 contagious isolates and 79.5 environmental isolates. As an overview, isolation in Summer and Autumn was significantly higher than Spring and Winter (**Table 3**).

The distribution of *Mycoplasma bovis* during all 4 four seasons was determined in 2022 and 2023. There were investigations on 45 large-scale dairy farms in 21 provinces of China, with collection of 1254 mastitis milk samples. Among them, 71.4% (15/21) provinces were identified as *Mycoplasma bovis* positive and 114 isolates were confirmed, with an isolation rate of 9.1% (114/1254). The positive rate of dairy farms was as high as 20.7% (30/145). The isolation rate of *Mycoplasma bovis* was higher in spring, summer and autumn (9.2, 10.8 and 8.9%, respectively) than winter (6.3%).

Table 1 Milk samples from bovine mastitis (n=3126) collected on 255 dairy farms in 22 provinces in China (2022 and 2023).

No	Area	Province	Farm	Milk sample	Contagious pathogen			Environmental pathogen							Others
					Staphylococcus aureus	*Streptococcus agalactiae*	*Mycoplasma bovis*	*Escherichia coli*	*Klebsiella spp*	*non-aureus Staphylococci*	*S. dysagalactiae, S. uberis*	*Nocardia*	*Serratia*	*Aerococcus viridans*	
1	Northeast	Heilongjiang	14	255	1	4	36	19	28	40	12	0	4	0	25
2		Jilin	3	10	0	0	0	2	0	2	3	0	0	0	5
3		Liaoning	9	147	5	9	5	9	37	20	12	0	5	1	28
4	Northern China	Beijing	7	43	0	0	2	10	8	8	8	0	0	0	6
5		Tianjin	13	133	1	0	4	7	18	30	14	0	4	0	31
6		Hebei	53	601	8	12	12	76	80	102	38	0	11	1	77
7		Inner Mongolia	31	348	5	11	4	60	23	66	31	0	2	0	66
8		Shanxi	1	10	0	0	2	0	0	0	0	0	0	0	0
9	Northwest and Southwest	Xinjiang	3	55	1	9	0	7	1	15	0	0	0	0	17
10		Gansu	14	186	9	5	2	23	35	37	9	0	3	0	26
11		Shaanxi	10	120	7	0	0	26	30	12	7	0	3	0	32
12		Ningxia	24	310	3	8	11	33	52	82	31	0	18	2	40
13		Yunnan	7	70	0	0	1	16	32	3	1	0	2	0	5
14		Sichuan	2	63	0	0	0	3	32	3	15	0	0	0	3
15	Eastern China	Shandong	21	280	5	4	12	29	32	116	18	21	4	1	48
16		Henan	3	25	5	0	15	3	4	8	1	0	1	0	6

17	Fujian	13	124	3	0	3	6	30	16	5	0	1	2	22
18	Hubei	2	15	0	0	3	3	5	0	0	0	0	0	0
19	Anhui	8	75	0	9	0	4	9	3	1	12	0	0	4
20	Jiangsu	4	56	7	6	0	2	6	10	13	0	0	0	4
21	Shanghai	4	68	6	2	0	5	32	6	19	0	1	3	3
22	Zhejiang	3	35	0	0	0	4	14	6	0	0	0	0	7
23	Guangdong	6	97	2	0	2	2	36	30	2	0	0	1	45
Total		**255**	**3126**	**68**	**79**	**114[a]**	**349**	**544**	**615**	**240**	**33**	**59**	**11**	**500**

[a]In total, 1254 milk samples were tested for *Mycoplasma bovis*.

Table 2 Distribution of selected 2612 pathogens isolated from 3126 mastitis milk samples in 4 regions of China collected from 255 large (> 500 cows) dairy herds in 2022 and 2023.

Pathogen	Northeast n=312		Northern n=838		Northwest & Southwest n=712		Eastern China n=750	
	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	6	1.9	14	1.7	20	2.8	28	3.7
Streptococcus agalactiae	13	4.2	23	2.7	22	3.1	16	2.1
Mycoplasma bovis	41	13.1[a]	24	2.9	14	2.0	35	4.7
Escherichia coli	30	9.6	153	18.3[a]	108	15.2[a]	63	8.4
Klebsiella spp.	65	20.8[a]	129	15.4[a]	182	25.3[a]	168	22.4[a]
Non-aureus Staphylococci	62	19.8[a]	206	24.6[a]	152	21.4[a]	195	26.0[a]
Streptococcus dysgalactiae and S. uberis	27	8.7	91	10.9	63	8.9	59	7.9
Nocardia spp	/	/	/	/	/	/	33	4.4
Serratia spp.	9	2.9	17	2.0	26	3.7	7	0.9
Aerococcus viridans	1	0.3	1	0.1	2	0.3	7	0.9

Other bacteria	58	18.6	180	21.5	123	17.3	139	18.5

[a]Within a row, percentage of pathogens without a common superscript differed ($P < 0.05$).

Table 3 Distribution of *isolation* of mastitis pathogens during all 4 seasons over 2 years in China.

Time	Spring[1]	Summer	Autumn	Winter
2022	242 isolates (291 samples, 27 farms, 13 provinces), 20 CP, 138 EP	391 isolates (457 samples, 45 farms 15 provinces), 38 CP, 218 EP	164 isolates (190 samples, 23 farms, 9 provinces), 33 CP, 104 EP	61 isolates (144 samples, 10 farms, 8 provinces), 15 CP, 15 EP
2023	673 isolates (567 samples, 44 farms, 18 provinces), 62 CP, 300 EP	778 isolates (524 samples, 47 farms, 13 provinces), 23 CP, 320 EP	1212 isolates (603 samples, 50 farms, 14 provinces), 46 CP, 346 EP	408 isolates (311 samples, 29 farms, 11 provinces), 14 CP, 144 EP
Average	915 isolates, 858 milk samples, 71 farms. Average of 457.5 isolations per year; 41 CP, 219 EP	1169 isolates, 981 milk samples, 92 farms. Average of 584.5 isolations per year; 30.5 CP, 269 EP	1376 isolates, 793 milk samples, 73 farms. Average of 688 isolations per year; 39.5 CP, 225 EP	469 isolates, 455 milk samples, 39 farms. Average of 234.5 isolations per year; 14.5 CP, 79.5 EP

[1]Spring: March –May; Summer: June – August, Autumn: September –November; Winter: December – February.
CP = contagious pathogens; EP = environmental pathogens

Discussion

Comparison milk samples collection provinces between 2022 and 2023

Data from each province of China, including milk samples and dairy herds, is in Figure 1. For the current study, milk samples were collected from 20 provinces of China in 2022 (**Figure 1A)**, but that increased to 22 provinces in 2023 (**Figure 1B).** In total, 1082 mastitis milk samples were collected from 86 large-scale dairy herds in 20 provinces in 2022, which included dairy cows in the main production area. The most represented provinces were Heilongjiang and Hebei, with each contributing >200 milk samples (**Figure 1A)**. By 2023, a total of 2044 milk samples had been collected from 169 large-scale dairy farms in 22 provinces across the country. Collections were nearly double 2022, with > 200 samples from each of 3 provinces (Inner Mongolia, Ningxia, and Hebei; **Figure 1B)**.

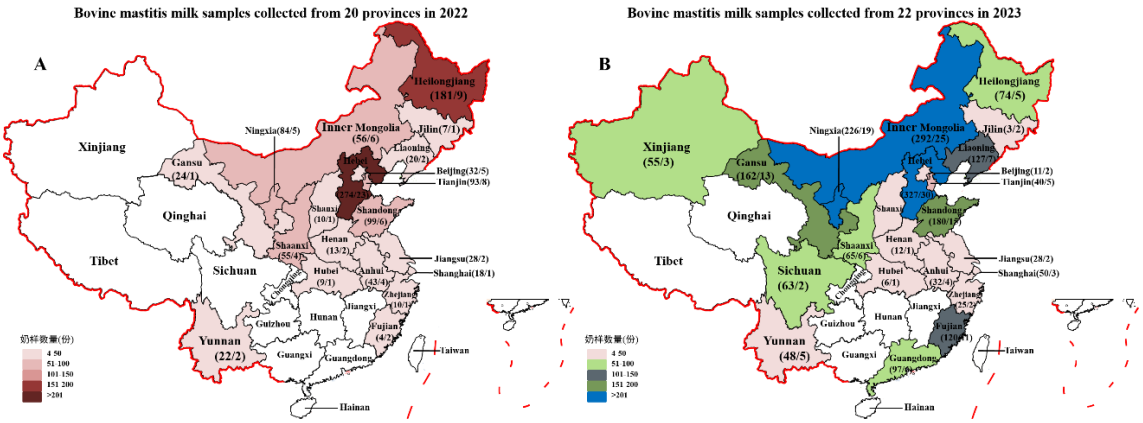
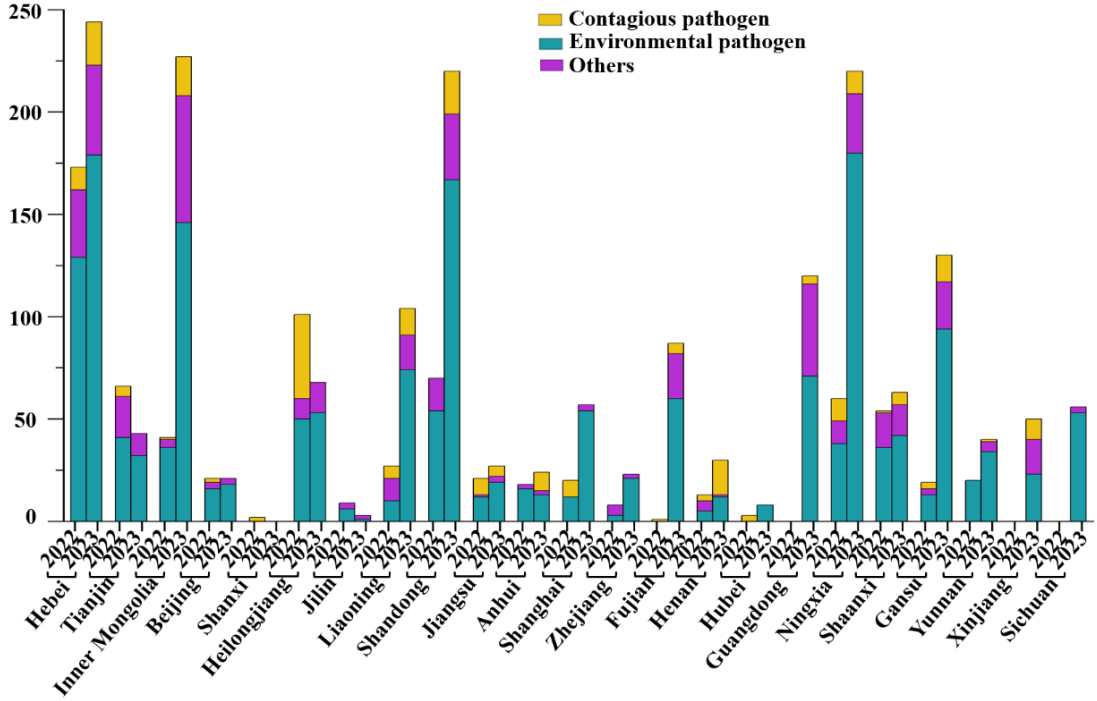


Figure 1 Milk sample collection for mastitis in dairy cows and herds in China (2022 and 2023).

The total number of dairy cows in China reached 10.5 million at the end of 2023, with ~ 5.8 million lactating cows and milk production of 41.97 million tons. Sample collection covered the main dairy cow production area, and it appeared that the data largely represented the national mastitis condition. This was the first report to use a large number of samples to characterize bovine mastitis in China. In previous studies, Gioia et al. (2021)(**6**) from America, Acharya et al. (2021) (**9**) from Canada and Ito Eleodoro and Fagnani (2023) (**4**) from Brazil used large numbers of milk samples to clarify the most frequent microorganisms associated with bovine mastitis. Analysis of large numbers of milk samples within a country should reflect bovine mastitis in that country.

In the present study, defined pathogens more than doubled from 2022 to 2023, with 747 and 1865 pathogens in these 2 years, respectively. The majority of isolates were environmental pathogens, followed by contagious pathogens, similar to Gao et al. (2017)(**10**), Ruegg (2017)(**11**) and Song et al. (2020) (**12**). Regarding the distribution of the pathogens among provinces, environmental pathogens were mainly isolated from Hebei, Inner Mongolia, Shandong, Ningxia and Gansu provinces in 2023, with significant increases compared to the same province in 2022 (**Figure 2**). However, pathogens in Xinjiang, Sichuan, and Guangdong provinces in 2023 had slight increases compared to other provinces.

Figure 2 Comparative analysis of milk samples pathogens isolated from bovine mastitis in various provinces in China in 2022 and 2023.



Comparisons to our team group investigation (published in 2017)

The current trend of national bovine mastitis pathogens distribution varied significantly compared to our team group national investigation published in 2017 (**Gao et al., 2017**)(**10**). Specifically, there was a remarkable increase in isolation rate of NAS, from 11.3 to 23.2% in the current study, and Klebsiella spp. from 13.0 to 21.1% in 2023, *Mycoplasma bovis* went from none in 2022 to 6.7% in 2023, and Bacillus spp. from 1.2% to 9.4% in 2023, Enterobacter spp. (from 5.5% in 2017 to 8.0% in 2023). In contrast, there were significant decreases in isolation rates of *S. aureus* (from 10.2% in 2017 to 2.3% in 2023) and *E. coli* (from 14.4 to 11.1% in 2023), and Environmental Streptococci (from 12.6 to 11.9% in 2023). However, rates for *Streptococcus agalactiae* and culture negative were relatively stable (**Table 4**).

Table 4 Comparison of current results and previous (2017) team group investigation.

Pathogen	2017 (%)	2023 (%)
Non-aureus Staphylococcus (NAS)	11.3	23.2
Environmental Streptococci	12.6	11.9
Streptococcus agalactiae	2.8	2.7
Staphylococcus aureus	10.2	2.3
Mycoplasma bovis	/	6.7
Klebsiella spp.	13.0	21.1
Escherichia coli	14.4	11.1
Enterobacter spp.	5.5	8.0
Bacillus spp.	1.2	9.4
Culture negative	15.8	14.7
Other	0.2	2.9
Mixed culture	3.6	7.6
Contaminated	3.8	3.5

Evaluation of advanced strategies for managing bovine mastitis

It was clear that environmental pathogens varied over time, but were currently the main etiologic agents of clinical mastitis in dairy cattle; they are regarded as closely related to management, including herd feeding and environmental control, including cleanliness and operation of barn cleaning equipment. It is recommended to improve cleanliness of cow resting areas, better manage feces, use pre- and post-milking teat dipping, and perform regular maintenance of milking equipment. The dry period is a high-risk stage for new infections with environmental pathogens. Several measures such as application of dry-cow preparations and sealants at drying off, management of dry milk herds, and risk assessment of mammary gland infections during the dry milk period should reduce the risk of infection. Notably, Gram negative bacteria such as Klebsiella spp. and *Escherichia coli* had a high prevalence; these pathogens can release endotoxins during infection, leading to serious inflammatory reactions. Therefore, there is a strong rationale to use nonsteroidal anti-inflammatory drugs and perhaps alternative medicine during treatment to reduce inflammation. Based on the varying clinical manifestations of pathogens, selective clinical mastitis treatments (**de Jong et al., 2023**)(**16**) and alternative treatments (**Li et al., 2023**) (**17**) can be practical tools to reduce antimicrobial use and alternative treatments on dairy farms. Ultimately, our results contribute to risk assessment of selective treatment and provided a baseline for setting and evaluating control measures and designing strategies to limit antimicrobial resistance.

In conclusion, there were trends of decreasing contagious pathogens and increasing environmental pathogens. Furthermore, there were also indications that selective and alternative treatment have promise to control bovine mastitis in China. Future research should address the environmental origin of mastitis-causing pathogens.

Statement of ethics

The study was reviewed and approved by the China Agricultural University Animal Care Committee and Standard Biosafety and Security Committee (Protocol SYXK, 2016–0008).

Funding

This work was supported in part by the National Natural Science Foundation of China (No. 31772813, 32273082 and U21A20262), the High-end Foreign Experts Recruitment Program (No. G2022108009L).

Acknowledgments

The author expresses his sincere appreciation to Prof Dr John P. Kastelic for reviewing and editing the manuscript. The author also appreciates our team group members for the huge work.

Conflicts of Interest

The author declares that he has no conflicts of interest that could have influenced the study.

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Vaccination against IBR & BVD; What to Consider?

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Introduction

Even with the importance of heard health on profitability, the question is often asked; can time and laborbe reduced by vaccinating animals at the start of the synchronization protocol or breeding season?

Modified-live virus (MLV) vaccines stimulate the immune system by actively infecting host cells. In general, these types of vaccines are considered to be more cross-reactive and broader in their immune system stimulation (antibody production and cell-mediated immunity), exhibit longer duration of effect; however, MLV vaccines also carry with them the potential to revert to virulence and inflict the damage they are designed to prevent. IBR gene deleted (marker) MLV vaccines had been demonstrated to be efficacious and safer (Raaperi 2015; Silva 2019). Inactivated virus vaccines (IVV) are safe to use in a wide variety of circumstances yet carry the general considerations that their effects are less broad and of shorter duration compared to MLV vaccines (Kelling, 2007). It is commonly thought that IVV provide someprotection against these viruses, but the same level of protection as a MLV is not achieved (Zimmerman et al., 2007; Rodning et al., 2010). However, a study that vaccinated heifers with a MLV prior to their first breeding season and then vaccinated with a Chemically Altered/Inactivated vaccine CA/IV before their second breeding season had similar levels of abortions following both a BVD and IBR challenge as animals vaccinated with a MLV before their second breeding season (Walz et al., 2017).

Infectious diseases affecting reproduction can create losses all throughout the reproductive process by decreasing ovulation rates, fertilization rates, embryonic survival rates, and fetal survival rates. Thus, thecow-calf industry spends millions of dollars a year to vaccinate cows against diseases that can impact reproductive efficiency. This is important as reproductive performance is of critical importance to the profitability in a cow-calf operation, but *the caveat to reproductive management is the things you do well do not compensate for the mistakes you make*. Instead, the mistakes you make cancel out all the thingsyou do well. Thus, to have optimal reproductive efficiency we need to evaluate the details and how theycan impact efficiency.

Infectious Diseases Affecting Reproduction:

Several diseases can have an impact on reproductive performance. They include Brucellosis, Leptospirosis, Vibriosis, Trichomoniasis, Bovine Viral Diarrhea and Infectious Bovine Rhinotracheitis. Allof these diseases can impact reproductive performance through decreased conception rates and embryonic/fetal losses. This review will focus on viral diseases that are usually vaccinated for annually.

Bovine Viral Diarrhea

Evidence of exposure to BVD virus is widespread in cattle throughout the world (estimated prevalence 15.74% of cattle; (Su et al., 2022)) worldwide, and thus it is considered endemic in the majority of the countries of the world. In the United States prevalence is much lower (usually estimated at <1%). For example, one study reported only 25 out of 4530 were persistently infected (PI; 0.55% of cattle), but there

was at least 1 PI animal in 5 of the 30 herds tested in the south-central US (16.7%; (Fulton et al., 2009)), and another study reported that 24 out of 7,544 stocker calves were PIs (0.32%; (Stephenson et al., 2017)). The reproductive effects of BVD; however, surpass its other effects in economic importance, when the occurrence of persistently infected animals is factored in.

The impact of BVD on reproduction depends on the stage of gestation in which the cow or heifer is infected. Early gestation infection results in low conception rates due to early embryonic death. Infection in mid-gestation may result in the formation of persistently infected calves, which occurs as a result of infection during a period of fetal development (roughly between 40 and 120 days of gestation) in which the fetus is differentiating its own cells from foreign materials. The result is a calf that has incorporated the virus into its own body and sheds high levels of virus persistently throughout its lifetime. Later infections may result in congenital defects, late-term abortions, or the birth of congenitally infected calves, which are weaker and more prone to illness than normal calves.

A recent study reported the impact of BVD exposure during the breeding season on reproductive success(Epperson et al., 2021). The presence of a transient infection during the breeding season reduced AI conception rates by 22% and breeding season pregnancy rates by 20%. The BVD virus is spread throughmany body fluids including saliva, respiratory secretions, and feces. The virus does not persist in the environment but can survive long enough to be transmitted via infected equipment, needles, and palpation sleeves.

Infectious Bovine Rhinotracheitis (IBR, “Red-nose”)

IBR virus is also termed BHV-1, or “bovine herpesvirus 1.” Being a herpes virus (in the same family as viruses causing cold sores in people), it has a propensity to become “latent” or dormant in nerve clustersin the throat area or lower spine and can be re-activated during times of stress. Because of this, any animal exposed to IBR in the past could potentially shed the virus to susceptible animals. IBR is shed and transmitted in nasal secretions and aerosols from infected animals. In addition to its effects on the respiratory tract, IBR virus affects reproduction by its effects on the ovaries, uterus, and developing embryo or fetus. The result can be infertility or early embryonic death, but in addition, IBR is one of the most frequently diagnosed viral causes of late-term (5^{th} to 9^{th} month of gestation) abortions.

Impact of Vaccination against IBR and BVD on Reproductive Performance

The effects of vaccination on estrus synchronization and conception are variable. A study in which the vaccination history was not reported and titer concentrations were not determined indicated that vaccination with a MLV at time of the start of a synchronization protocol (day -9, with AI on day 1 to 5) didnot impact estrous response or pregnancy success (Stormshak et al., 1997). In another study, animals were vaccinated with a MLV vaccine at least two times prior to synchronization protocol (the second dosebeing administered at day -90 prior to peak breeding day). The heifers were then revaccinated either at - 40 d or -3 d prior to peak breeding (three doses total) and no differences in conception rates were observed (Bolton et al., 2007). However, several studies have reported negative impacts on pregnancy success by vaccinating naïve heifers with a MLV around time of breeding (Miller et al., 1989; Chiang et al., 1990; Miller, 1991; Perry et al., 2013).

Vaccination at the start of the breeding season: Most recently developed estrous synchronization or fixed-time AI protocols in heifers and cows try to control follicular development by inducing ovulation at the start of the synchronization protocol; therefore, insemination should occur on the second ovulation after the start of the protocol (Lamb et al., 2010; Grant et al., 2011). To investigate if vaccination only impacted the follicular wave present at the time of vaccination, naïve heifers were vaccinated with either aMLV or IVV at the time of the first induced ovulation of a fixed-time AI synchronization protocol (Perry et al., 2013). In this study, no control heifers (nonvaccinated) experienced an abnormal estrous cycle following AI. An abnormal estrous cycle was defined as an estrous cycle less than 15 d (concentrations of P4 decreased to < 1 ng/mL prior to day 15 after AI) or concentrations of P4 never increased above 1 ng/mL. Heifers vaccinated 36 and 8 days before AI with an IVV experienced 10% (2/21) abnormal cyclesand heifers vaccinated 8 days before AI with an IVV experienced 14% (1/7) abnormal cycles. There was no difference between these groups (P = 0.72), and both were similar to controls (P = 0.31 and 0.22, respectively). A greater percentage of heifers vaccinated with a MLV 8 days before AI had abnormal estrous cycles [38% (8/21)] compared to control heifers (P = 0.02). Of the heifers that experienced an abnormal estrous cycle, 100% of heifers in both IVV groups (2/2 and 1/1) conceived during the return cycle. However, only 38% of heifers vaccinated with a MLV (3/8) conceived during the return cycle.

Table 1. Impact of vaccine on luteal function and pregnancy success in naïve animals.

Vaccine	Abnormal luteal function	AI Pregnancy Success (%)	Pregnancy Success (%) to second service
1 dose Modified Live	8/21 (38%)[b]	7/21 (33%)[b]	3/8 (38%)
1 dose Inactivated	1/7 (14%)[a]	5/7 (71)[ab]	1/1(100%)
2 doses Inactivated	2/21 (10%)[a]	17/21 (81%)[a]	2/2 (100%)
Saline	0/10 (0%)[a]	9/10 (90%)[a]	-----

Means within a column having different superscripts are different [ab]P < 0.05
Adapted from Perry et al., 2013

In previously vaccinated cattle, a study was conducted to examine the differences in pregnancy success between beef females vaccinated with either a MLV vaccine or an IVV vaccine 30 days before the breeding season, with sufficient power to detect a difference of less than 10 % in pregnancy success between groups (9 herds with 1436 animals) (Perry et al., 2016). Conception rates to the fixed-time AI tended to differ between MLV treated animals and IVV treated animals (*P* = 0.055), but control animals were intermediate with no difference in conception rates between MLV and Control (*P* = 0.21) or betweenIVV and Control (*P* = 0.49). When pregnancy was determined on day 56 of the breeding season (AI conceptions plus 1 return estrus) conception rates in the IVV group were greater (*P* = 0.01) compared to the MLV group. Animals treated with MLV also had decreased pregnancy success compared to the Control (*P* ≤ 0.01), but there was no difference between IVV and Control. Following the breeding season, pregnancy success was similar between MLV and Control (*P* = 0.34) as well as between the Inactivated and Control (*P* = 0.14), but there was still a difference between MLV and IVV (P = 0.01). a second field study was conducted to examine the differences in pregnancy success between beef females vaccinatedwith either a MLV vaccine or a CA/IV vaccine between 27 and 89 days before the breeding season, with sufficient power to detect a difference of less than 10 % in pregnancy success between groups (10 herdswith 1565 animals) (Perry et al., 2017). Conception rates to AI were greater in the CA/IV vaccine group compared to the MLV vaccine group (*P* = 0.05; 60% vs 52%; Table 2).

Table 2. Impact of vaccine on pregnancy success among previously vaccinated animals.

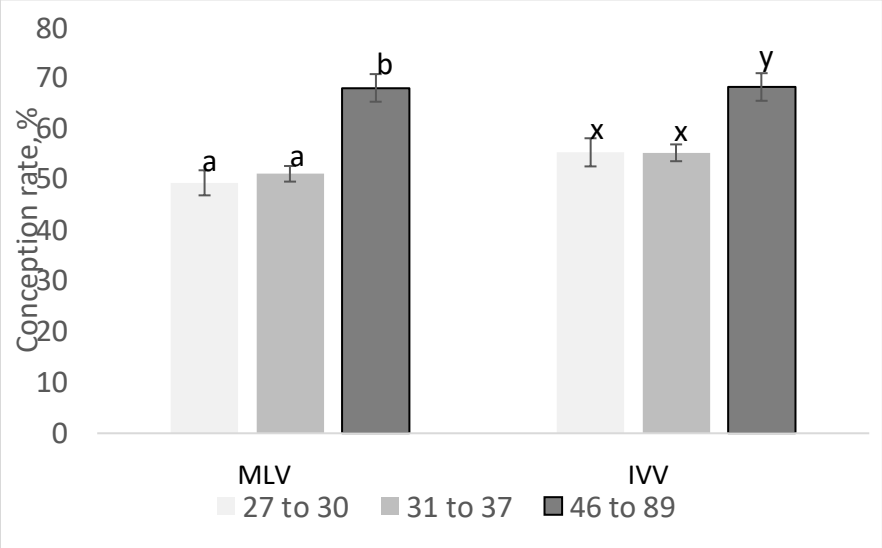
	Vaccine	AI Conception (%)	Day 56 Pregnancy Success (%)	Breeding Season Pregnancy Success (%)	Early Embryo Loss (%)
Study 1	Modified Live	40.0 ± 4^{a}	88.9 ± 2^{c}	95.2 ± 2^{c}	2 ± 1
	Inactivated	46.5 ± 4^{b}	93.2 ± 2^{d}	98.0 ± 1^{d}	2 ± 1
	Saline	43.3 ± 4^{ab}	92.5 ± 2^{d}	96.4 ± 1^{cd}	2 ± 1
Study 2	Modified Live	52.0^{y}		95.2 ± 2	
	Chemically Altered/Inactivated	60.0^{z}		96.4 ± 1	

Means within a column having different superscripts are different $^{ab}P = 0.055$, $^{cd}P \leq 0.01$, $^{yz}P < 0.05$
Adapted from Perry et al., 2016 and Perry et al., 2017

A recent publication (Stewart et al., 2023), reported that when cows that had previously been vaccinated were vaccinated at the start of the synchronization protocol that animals vaccinated with a MLV had increased AI conception rates compared to cows vaccinated with an IVV in a spring breeding season (54% vs 46%; P< 0.01), but in a fall breeding season AI conception rates did not differ (P = 0.62; 48% vs 49%, respectively). It is unknown why there were differences between spring and fall, but some differences between this study and the previously mentioned study include, a different MLV vaccine was used, and all cows were vaccinated only 10 days before AI. In addition, there was not a nonvaccinated control group or blood samples collected to determine possible exposure to wildtype viruses. Thus, it cannot be determined if differences between treatments and season could be due to protection or timing. A recent study reported that when naïve heifers were vaccinated at the start of a synchronization protocol with a MLV control animals housed in the same pen seroconverted before the end of the study (Chase unpublished data). This indicates that when animals are vaccinated with a MLV animals can shed the virus impact animals that they are around

Timing of vaccination

Negative impacts of vaccinating with a MLV on pregnancy success has been reported on not only on first service conception rates, but also on a low percentage of animals conceiving during the second service following vaccination (Chiang et al., 1990; Perry et al., 2013), and in some heifers infected with BHV-1 at or near estrus, normal estrous cycles were delayed for up two months (Miller and Van der Maaten, 1985).Furthermore, BVDV antigen has been detected in the ovary up to 30 d post-vaccination (Grooms et al., 1998). In the second field trial mentioned above, interval from vaccination with either MLV or IVV until AI also influenced conception rates (P = 0.02; Figure 1). Animals vaccinated 27 to 30 d prebreeding and animals vaccinated 30 to 37 days prebreeding had similar (P = 0.98; 52% and 52%) conception rates; however, both were decreased compared to animals vaccinated 38 to 89 d prebreeding (P < 0.03; 64%). There was no treatment by interval interaction (P = 0.79), indicating at all three intervals conception ratesto the CA/IV vaccine were increased compared to the MLV. Furthermore, there was no effect of treatment (P = 0.18) or treatment by interval interaction (P = 0.17) on breeding season pregnancy rates.



Possible causes of decreases in reproductive performance

Decreases in fertility by vaccination of naïve heifers around the onset of standing estrus are likely mediated through negative effects on corpus luteum (CL) function (Van der Maaten and Miller, 1985; Smith et al., 1990), with the hypothesis that the virus can get inside antral follicles and disrupt the formation and development of the corpus luteum. This has further been established as vaccination of naïve heifers with a MLV around time of breeding has negative impacts on corpus luteum development and on pregnancy success (Miller et al., 1989; Chiang et al., 1990; Miller, 1991) even when utilizing a synchronization protocol that induces ovulation of the dominant follicle at the start of the protocol (Perry etal., 2013).

The same effect of abnormal appearing corpora luteal (no signs of necrosis or mineralized luteal tissue) did not occur when heifers had been vaccinated twice with a IVV and then vaccinated with a MLV, but 37.5% (3/8) of animals that exhibited estrus had a large CL with progesterone concentrations less than 1ng/mL on day 6 to 7 after estrus (Spire et al., 1995). The mechanism that inflicts CL damage following MLV vaccination is still unknown. Our laboratory has recently investigated the effect of a commercially available MLV or IVV vaccine administered around the time of estrus on CL development and function through evaluation of luteal cell populations, degree of apoptosis, and circulating progesterone and cytokine concentrations (Epperson, 2023). There were reduced numbers of large luteal cells (LLC) in MLV compared to IVV and controls (P<0.0001), but IVV were similar to controls (P=0.11; Table 3). MLV

had a decreased percentage of LLC compared to controls, and IVV were intermediate (P<0.0001, MLV: 1.57±0.33%, IVV: 2.99±0.30%, Control: 6.45±0.33%). Based on P4 concentrations, 24% of MLV and 0% of IVV had an abnormal cycle following vaccination. Overall, MLV had reduced P4 concentrations (P=0.02; MLV: 3.61±0.22; IVV: 4.81±0.46ng/mL). The new CL that formed following an abnormal cycle in MLV had the greatest percentage (35.56 ± 5.5%) apoptotic cells. Treatment by cycle status interaction, and time significantly affected IFN-γ, IP-10, MIP-1β, and MCP-1 (P < 0.03), with several time points having elevated concentrations in abnormally cycling MLV animals. Collectively, this demonstrates MLV vaccination around estrus negatively influenced luteal cell populations, P4, and increased luteal apoptosisand pro-inflammatory cytokines.

Table 3. Histological and Apoptotic Evaluation of the Corpus Luteum.

	MLV[a]-new CL[b]	MLV-old CL	IVV[c]	Control	*P*-value
Large luteal cell number	5.11 ± 0.86^{x}	3.74 ± 0.73^{x}	12.33 ± 0.79^{y}	14.22 ± 0.86^{y}	< 0.0001
Total cell number	342.69 ± 15.72^{x}	339.51 ± 13.28^{x}	451.73 ± 14.35^{y}	233.34 ± 15.72^{z}	< 0.0001
Large luteal cell, %	1.57 ± 0.33^{x}	1.24 ± 0.28^{x}	2.99 ± 0.3^{y}	6.45 ± 0.33^{z}	< 0.0001
Luteal apoptosis, %	35.56 ± 0.06^{x}	7.055 ± 0.05^{y}	4.248 ± 0.05^{y}	--	< 0.0001

[a]modified-live virus vaccine (MLV)
[b]Corpus Luteum (CL)
[c]Inactivated virus vaccine (IVV)
Different ^{xyc}superscripts within rows depict statistical differences ($P \leq 0.05$) between treatments.

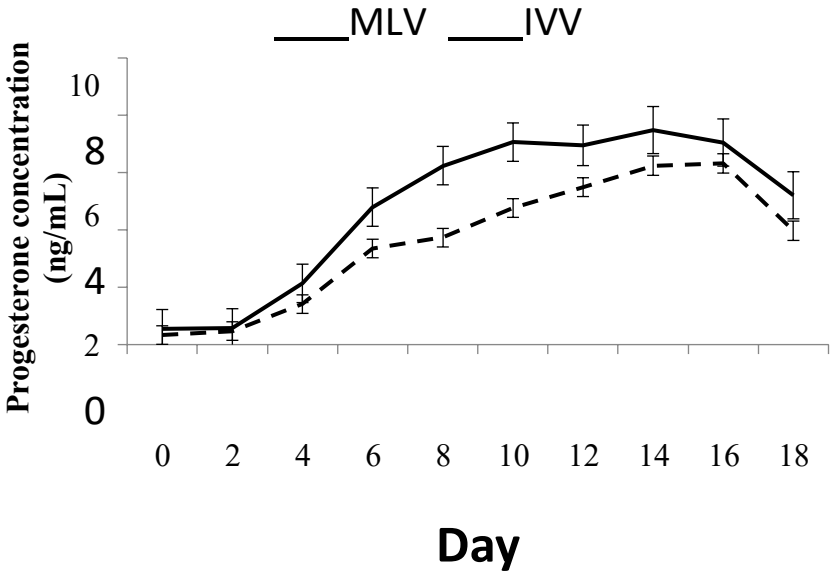


Figure 2. The influence of the interaction of treatment and time on circulating concentrations of progesterone (ng/mL) among all bovine females administered either a modified-live virus vaccine (MLV) or inactivated virus vaccine (IVV) on d 0 (P = 0.05). Data points marked with a *superscript denote statistical differences ($P \leq 0.05$) between treatments within day, while those marked with a #superscript tend to be different ($0.05 < P \leq 0.10$)

Conclusions

So where do these studies leave us on the impact of virus vaccines on reproductive success? Vaccines against infectious reproductive diseases are valuable tools in the prevention of these diseases, as outbreaks of these diseases can be potentially devastating to a beef herd. This emphasizes the importance of proper vaccination of females before they enter the breeding herd.

However, evidence is growing that MLV versions of these vaccines can have negative effects on reproductive management in well managed herds. Studies utilizing different pre-breeding vaccination protocols and intervals indicate that MLV vaccines, when given at labeled pre-breeding intervals, may negatively affect reproductive parameters compared to cattle vaccinated with inactivated vaccines. In light of this research, it appears the choice of pre-breeding vaccine product type and timing is one to carefully consider. Important to this consideration is the level of exposure that a given herd may have, as none of these large prebreeding studies were carried out in the face of disease challenge and do not address the question of protection in the face of an infectious reproductive disease exposure. Future research will help determine how to strike the best balance between appropriate disease protection andminimizing harmful effects from the vaccines themselves. It is reasonable to expect that striking this balance will be different for each individual cattle operation, making it imperative that cattle producers consult their veterinarian and weigh all available information when making decisions about pre-breedingvaccinations in their herds.

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The experience of BVD & IBR control in Europe; how it started and where are we?

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Objective

This presentation focuses on IBR and BVD control and eradication in Europe, referring to the historical experience on control addressing the subject since the early 1970s until these diseases became regulated, after 2021, with the intention of eradicating them from European Union territory. It will also discuss the fundamentals of control and eradication programs at regional and national levels but will not forget to focus on private schemes and their importance and utility for the economy of cattle herds, when country level control initiatives driven by official authorities are lacking.

Bovine Herpesvirus 1 (BHV-1) and 2 (*Alphaherpesvididae*) are causative agents of Infectious bovine rhinotracheitis / Infectious pustular vulvovaginitis (IBR) was first reported in cattle in the USA during the 1950's. IN Europe Council Directive 64/432/EEC, in 1964, listed IBR as "*a disease to which every member state could receive approval for a national IBR control program for its entire territory or a part of it*". First IBR control programs initiated in Europe during 1970's, Scandinavian countries being the first to become officially free followed by Austria and oarts of Italy. Bovine viral diarrhea (BVD) is caused by bovine viral diarrhea virus BVDV (genus *Pestivirus*), of which there are mainly BVDV-1 or BVD-2 types present in bovine population. BVDV is responsible by BVD and mucosal disease (MD) complex, MD being caused by combination and cytopathic with non-cytopathic BVDV superinfection, often driven by PI animals presence.

IBR and BVD are diseases of the list B of the Animal Health Law, the EU Regulation 2016/429, from the Parliament and the Council. Since April 2021 these diseases are formally recognized as category B diseases (under Regulation 2018/1882), meaning "*listed diseases which must be controlled in all Member States with the goal of eradicating"* it throughout the Union, as referred to in Article 9(1)(b) of Regulation (EU) 2016/429. Currently there are 24 IBR and 23 BVB control programs (CP's) running in European Countries, part of them compulsory and most of them at national level. Both IBR and BVD are responsible for significant losses associated with clinical and subclinical disease, death, abortion, reproductive impairment, and costs linked to treatment, and control.

Although these diseases are often addressed together in vaccination schemes or in control and eradication programs, they have very different epidemiology which determines different key aspects of control and eradication. In brief, IBR generates lifelong latent carriers which play a key role in introduction and spread of the virus in herds and in its maintenance, until they are removed from herds. After infection an animal can be considered infected for life, shading periodically the virus. Latent and chronic animals are often difficult to detect with serology, and vaccination programs based in hyperimmunization are efficient in reducing viral excretion, hence cutting the spread of the virus within the herd. Hyperimmunization programs can bring R_0 values within herds to near zero. The virus can be eliminated from a herd if vaccination programs persist long enough, and providing infected animals are removed in final stages of control.

Regarding BVD, persistently infected (PI) animals play a key role- PI animals result from fetus infection *in utero* (between 80 to 130 days of gestation), are animals that may survive for long time in the herds: they often become producing cows giving birth to PI offspring or breeding bulls. PI animals act as permanent supers-shedders, spreading large amounts of virus in the farm environment and maintaining the infection in the herd. The PI animals cannot be vaccinated because they do not recognize BVD as an antigen and vaccines are not efficient to stop viral shedding from these animals. Vaccination in infected herds, is essential, aiming at avoiding PI formation by protecting pregnant mothers, and also to prevent clinical disease during viral circulation episodes. Eradication of BVDV lies on detection and elimination of PI animals from the herds coupled with vaccination programs.

At the basis of all control and eradications schemes for IBR or BVD there is i) individual animal identification, ii) definition of *herd status* with, at least two categories: free herd, and infected herd, and iii) control of animal movement. Additionally, control programs for IBR relay in mass vaccination with DIVA vaccines (Differentiating Infected from Vaccinated Animals), usually gE deleted and their complementary tests. Elimination of positive animals needs to be considered, especially at the end of the program completion both at farm and at national level. Regarding BVD, control programs focus on PI detection and elimination, and vaccination especially focusing the protection of the reproductive herd to prevent PI formation and clinical and subclinical co-morbidities. The concept of DIVA vaccine towards BVDV has not so far been achieved; however the use of vaccines which can allow to differentiate vaccinated from previously infected animals is key to monitor efficiency of control or identify viral entrance in free farms. Live vaccines for BVD are difficult tools to manage in eradication because they make serological tests "*blind*"; nonstructural protein p80 is usually in EIISA tests as a proxy to DIVA concept but has a few drawbacks. Other key elements at farm level, for both diseases, are Biosecurity measures aimed to prevent the virus entrance and to spreading in free farms; these need to be coupled with monitoring schemes designed to assess the maintenance of the farm virus free status or to monitor progress of eradication by assessing different target groups within the farm.

The example of a voluntary program led by farmers organizations in Portugal, named Bovicontrol, will be briefly discussed as a positive example of how the industry, farmers and veterinarians can work together to tackle successfully these diseases.

To conclude, given the relevance of IBR and BVD control to the economy of highly efficient dairy or beef farms it is worthwhile to embrace control programs at farm level. The European experience, after more than 50 years, shows that it is possible to control and eradicate IBR and BVD and that the programs are cost effective. Consequently, the adoption of herd specific testing and vaccination schemes at proves to be cost effective even in countries without official schemes.

Inflammatory responses in tissue and mucus of reproductive tract in periparturient cow

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A healthy reproductive cycle is necessary to improve the productivity in cattle, and this requires normal pregnancy progress and calving as well as fertility, and structural and functional recovery of the postpartum tract. This article focuses on the inflammatory response of the reproductive tract from the last trimester of pregnancy to the postpartum period.

Inflammatory responses during cervical ripening in the prepartum period Several factors can reduce productivity in cows, but dystocia accounts for the majority of the parturition-related abnormal conditions and causes significant economic losses. Dystocia leads to stillbirths and newborn deaths, and about 70% of all fetal deaths are due to dystocia. It is estimated that producers lose more than $75 million a year in their income throughout Japan. In addition, the causes of dystocia are closely related to foetal overgrowth and birth canal abnormalities. The recent increase in the number of cows with abnormalities of the birth canal has become a problem. This is due to prolonged gestation or fetal overgrowth, which is due in part to insufficient cervical ripening.

Cervical ripening is thought to be a local inflammatory response without infection and caused by the presence of macrophages that stimulate the production of inflammatory cytokines, which act on blood vessels, causing inflammatory cells to migrate and degrade the extracellular matrix, type I collagen, resulting in softening and ripening of the cervical canal. The presence of macrophages has been suggested to be important in cervical ripening in humans and mice. In humans, macrophage infiltration is at least doubled during labor
(Sakamoto *et al*., 2005), and in mice, cervical ripening does not progress when cervical macrophages are artificially reduced (Yellon *et al*., 2019). Pro-inflammatory M1 macrophages increase and anti-inflammatory M2 macrophages decrease in cervical tissue as parturition approaches in rats (Jinying *et al*., 2019). In addition, various inflammatory cytokines have been implicated in cervical ripening. In humans, the concentrations of inflammatory cytokines such as
interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α in amniotic fluid
increased before delivery (Garcia-Velasco *et al*.,1999; Keelan *et al*., 1999, 2003; Young *et al*., 2002), and increased IL-1β, IL-6, and IL-8 in the uterus contribute to the process of cervical dilation (Winkler *et al*., 2001; Sakamoto *et al*., 2004). In guinea pigs, direct application of IL-8 to the cervix is known to soften the cervix
(Chwalisz *et al*., 1994). It has also been observed that a decrease in type I collagen coincides with the softening of the cervical canal in mice (Yan *et al*., 2019).

However, the details of the cervical ripening process in cattle are unknown, with only a report of neutrophil infiltration of cervical tissue and increased IL -8 expression from the last trimester of pregnancy to parturition. Since there is no report on the dynamics of cytokines and neutrophil infiltration of cervical mucus secreted from the cervical canal before parturition, and since cervical mucus is easier to collect than tissue biopsy, we focused on cervical mucus as well as cervical tissue

in our experiment s. We observed changes in type I collagen, macrophages, and mRNA expressions of IL-1α, 1β, 6, 8, and 10 and TNF-α in the cervical tissue, and neutrophil infiltration and various cytokine dynamics in the cervical mucus from late pregnancy to calving to clarify one aspect of the normal cervical ripening mechanism in cattle. Cervical mucus and biopsy samples were collected from cows from 200 to 274 days of pregnancy and at 7-day intervals thereafter. Cervical mucus was collected directly by inserting the hand into the vagina. After smear preparation, the PMN%, or percentage of polymorphonuclear neutrophils over all nucleated cells such as endometrial epithelial cells in cervical mucus was calculated as an indicator of inflammation. Cervical mucus was extracted for protein in PBS, and then the protein concentration was measured using the NanoDrop spectrophotometer. The concentrations of various cytokines were measured using MAGPIX, a multi-parameter simultaneous analysis system, and the concentration of each cytokine was divided by the protein concentration to be used as the measurement value. For the biopsy samples , sectioned specimens were prepared and subjected to multiplex fluorescent immunostaining using anti-Iba-1 (all macrophages), anti-iNOS (M1-macrophage), and anti-CD163

(M2-macrophage) antibodies, and the macrophage infiltration rate was calculated. Total RNA was extracted from the cervical tissue and mucus, and the ratio of mRNA expressions of IL-1α, IL-1β, IL-6, IL-8, IL-10, and TNFα at 200 days of gestation to the other segments were analyzed.

In the cervical tissue, type I collagen decreased by 274 days in pregnancy, or 3 weeks before the due date. Macrophage infiltration was not observed 12 –14 weeks before the week of calving but was observed 5 –6 weeks before calving. There was a strong positive correlation between the expression sites of Iba -1 and

iNOS. Additionally, IL-6 mRNA expression in cervical tissue increased 3 weeks before calving. In the cervical mucus, there was an increase in PMN% as the week of fetal delivery approached. The mRNA expression of IL-1α increased 3 weeks before and during the parturition week, while that of IL -8 mRNA increased 2–3 weeks before and during the parturition week. The IL-1α, IL-1β, IL-8, and TNFα concentrations increased 0–3 weeks before calving as compared to those at 12–14 weeks before calving. These results indicate that cervical ripening begins 5–6 weeks before parturition in cows when M1 macrophages infiltrating the cervical tissue produce large amounts of IL-6. Moreover, the inflammatory cells infiltrate the cervical mucus, and IL-1α, IL-1β, IL-8, and TNFα levels increase toward parturition. The infiltration of M1 macrophages in bovine cervical tissues and the timing of increased expression of IL-6 mRNA suggest that M1 macrophages produce inflammatory cytokines, mainly IL -6, during cervical ripening in cattle.

Since IL-6 is an important factor in the so-called cytokine storm and is considered to trigger a local inflammatory response, the presence of M1 macrophages may trigger cervical ripening. In contrast, there was no change in IL-6 mRNA expression in cervical mucus, while there was an increase in IL-1α and IL-8 mRNA expression. This was consistent with changes in inflammatory cytokine concentrations in cervical mucus. In particular, IL-8 showed a marked increase in both mRNA and protein concentration. The increase in PMN% in cervical mucus also suggests that the inflammatory response in the cervical mucus is driven by the increase in IL-8 and the neutrophils mobilized by this IL-8. These inflammatory cytokines may also act on blood vessels to infiltrate inflammatory cells into tissues and promote the production of additional inflammatory cytokines. M1 macrophages work only in tissues, as IL-6 was not altered in mucus. Inflammatory cells and cytokines migrate with mucus into the cervical lumen, increasing inflammatory cytokines, mainly IL-8, in the mucus and promoting the migration of neutrophils.

These cytokines exert an external effect on the cervical tissue, which is suggested to play a role in decreasing type I collagen. Given the increased mRNA expression and concentration changes

observed in our study, it is likely that IL-8 in cervical mucus plays an important role in the local inflammatory response in the final stage of parturition in cattle.

Inflammatory cytokine profiles in lochia and its association with f ertility During calving, bacteria are introduced externally , and a high percentage of cows remain infected during the puerperal period. In healthy postpartum cows, physiological inflammation and the immune response expel the bacteria from the uterus, and uterine involution occurs. However, several factors, such as decreased immune competence in the host and growth of pathogenic bacteria , can affect normal uterine involution. Consequently, this exposes the uterine environment to pathological conditions and causes differing levels of endometritis . Failure to utilize the proper diagnostic tools to diagnose this condition can lead to
delayed time to conception.

Cow's lochia is derived from residual fetal fluid, postpartum hemorrhage, and shed placental tissue, and changes in colo ur and appearance from a few days to about two weeks after parturition. In the very early postpartum period, observation of lochia is useful for the subsequent development of clinical diagnosis of uterine diseases in cows. However, information on inflammatory response in this period and characteristics of lochia in the cow is scarce. We aimed to clarify the association of cytokine level and the presence of pathogenic bacteria in lochia, with the development of endometritis and fertility in dairy cows. Lochia samples
were collected by hand from 74 Holstein cows on 2, 5, 9, and 16 days postpartum (pp), and endometrial samples were collected at week 5 pp (W5) at a dairy farm in Oita Prefecture, Japan. A diagnosis of subclinical endometritis was made according to the cut-off point of 6 of endometrial PMN% at W5 (Dubuc *et al*., 2010) and the cows were divided into those with (affected group) and those without endometritis (non-affected group). Expression of cytokine mRNA in lochia and endometrial samples was determined, and the DNA copies of bacteria, which are known to be pathogenic to the endometrium, were determined on days 9, 16, and W5. Pregnancy diagnosis was conducted by rectal palpation 45 to 55 days after the first service pp. The results showed that in the non-affected group, the mRNA
expression of IL-1α, IL-1β, and TNF-α decreased from 9 to 16 days pp. In the endometritis-affected group, however, an increase in IL-1α, IL-1β, IL-6, and TNF-α expression was observed from 2 to 16 days pp. Regarding cytokine expression at 5 weeks postpartum, IL-1α, IL-1β, and IL-8 were significantly higher in the endometritis-affected group than the non-affected group. On the other hand, the expression of IL-10, an anti-inflammatory cytokine, tended to be higher in the non-affected group than affected group. In a comparison between cows that became pregnant by first AI pp (pregnant group) and those that did not (non-pregnant group) regarding cytokine expression in lochia from 2 to 16 days pp, a transient increase was observed at 9 days pp followed by a decrease by 16 days pp for IL-1α, IL-1β, and IL-8 in the pregnant group, whereas there was an increasing trend in these cytokines from 2 to 16 days pp in the non-pregnant group. Taken together, the above results indicate that inflammatory cytokine profiles from day 5 to 9 pp may serve as an indicator for evaluating the risk of subsequent development of endometritis and fertility in dairy cows.

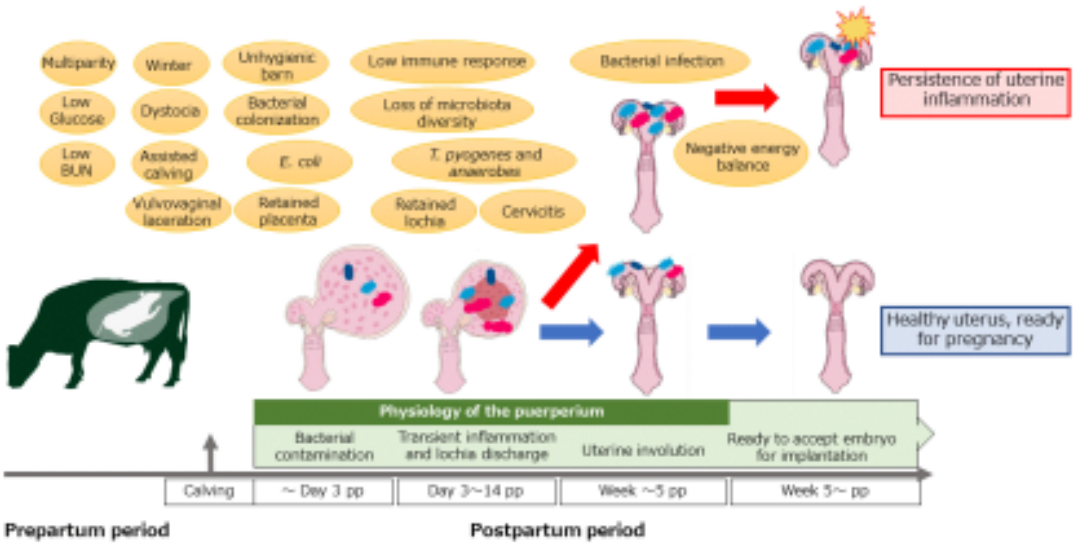

Figure 1. Predisposing factors for inflammation of the endometrium in the periparturient period in cows (Osawa, 2021)

Inflammatory responses during uterine involution in the postpartum period The process of uterine involution in the postpartum cow is that bacteria invade the uterus from the outside (environment) at calving, and in most cows , the bacteria are in a state of "contamination" (Figure 1). In healthy cows, physiological inflammation and immune response result in the expulsion of lochia during this period, while at the same time, the uterus decreases in size, elimination of bacteria, and uterine involution are completed. However, several factors, such as decreased immune competence in the host and growth of pathogenic bacteria, can affect normal uterine involution. Consequently, this exposes the uterine environment to pathological conditions and causes differing levels of endometritis. In general, the diagnosis of endometritis in clinical practice in cows is made after 5 weeks postpartum, and treatment is also delayed until later, thus contributing to delayed conception, i.e. prolonged open (n on-pregnant) period. Failure to utilize the proper diagnostic tools to diagnose this condition can lead to delayed time to conception. To improve bovine reproductive performance, research has been performed to elucidate the postpartum uterine environment, including bacterial flora, and changes in transient endometrial inflammation in healthy as well as diseased cows. *E. coli* and *Trueperella pyogenes* cause persistent infection (Ghanem *et al*., 2015). Approximately 30% of open cows have subclinical endometritis (SE) during the postpartum period (LeBlanc, 2008), and dairy cows with low blood glucose during prepartum have a high risk of developing SE (Ghanem *et al*., 2016). Moreover, cows with purulent vaginal discharge do not always have endometritis but only vaginitis and/or cervicitis (LeBlanc *et al*., 2011).

Although beef cows generally have a lower PMN% and a lower incidence of uterine disease than dairy cows, PMN% at 30 days pp was higher in animals that failed to conceive at first insemination than those that conceived. Additionally, PMN% remained below 10% ten days or later before insemination in all pregnant cows . Therefore, endometrial PMN% is a useful parameter in making breeding decisions, especially when expensive semen or embryo is used.

As for the treatment of endometritis, antibiotics have been used regardless of whether endometritis is clinical or subclinical. However, recent concerns about the emergence of drug-resistant bacteria have led to the search for alternatives to antibiotics. One of these is an antiseptic called polyvinylpyrrolidone-iodine (PVP-I).

It has long been used in veterinary practice, but there are mixed reports on its effectiveness. This is partly due to its administration regardless of accurately ascertaining inflammatory status at the time

of treatment. With the availability of cytobrush, it has become possible to objectively evaluate the level of endometrial inflammation and compare the intrauterine environment before and after administration. We have shown the effectiveness of 2% PVP-I in treating severe endometritis. Intrauterine infusion of PVP-I improved fertility and promoted endometrial epithelial cell regeneration after inducing transient uterine inflammation, suggesting that PVP-I could be a suitable alternative to antibiotics.

Conclusions

The series of physiological inflammatory responses during the periparturient period play an important role in the process of cervical ripening, normal calving, and uterine involution in the postpartum period.

Acknowledgments

The research works were supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) and Livestock Promotional Subsidy from the Japan Racing Association.

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Metritis in dairy cattle: fighting an old disease with new knowledge.

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This talk will deliver information on recent updates on methods for sampling and handling samples for BRD diagnosis and the surveillance of bovine respiratory disease (BRD) in dairy cattle. Evidence-based data from approaches used to collect samples for BRD pathogen diagnosis, as well as recent (last 2 years) data on studies comparing and evaluating in more depth methods for deep nasopharyngeal sampling, will be discussed. The impact of transport medium, temperature, and time on the recovery of BRD samples collected for some of the most common BRD bacterial pathogens observed in cattle will be discussed. We will also review the use of antibiograms in veterinary medicine, using BRD as a case example. Preliminary findings from an ongoing longitudinal project evaluating the use of an antibiogram framework for commercial dairy farms for BRD bacterial pathogens will be presented, and practical on-farm considerations will be discussed. Together, the information presented will provide practical information on sampling, diagnosis, and monitoring of BRD, as well as new concepts to consider when evaluating antibiotic resistance for BRD at a herd level.

Parasitic diseases

The Economic Impact of Parasites in Beef Cattle

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Abstract: Global human population growth requires consumption of more meat such as beef to meet human needs for protein intake. Cattle parasites are a constant and serious threat to the development of the beef cattle industry. Studies have shown that parasites not only reduce the performance of beef cattle, but also negatively affect the profitability of beef agriculture and have many other impacts including contributing to the production of greenhouse gases. In addition, some zoonotic parasitic diseases may also threaten human health. Parasitism challenges profitable beef production by reducing feed efficiency, immune function, reproductive efficiency, liveweight, milk yield, calf yield, carcass weight and leads to liver condemnations and disease transmission. Globally, beef cattle producers incur billions (US$) in losses due to parasitism annually, with gastrointestinal nematodes (GIN) and cattle ticks causing the greatest economic impact. Appropriate use of anthelmintics, endectocides and acaricides have widely been shown to result in net positive return on investment. Implementing strategic parasite control measures, with thorough knowledge of parasite risk, prevalence, parasiticide resistance profiles, and prices can result in positive economic returns for beef cattle farmers in all sectors.

Keywords: beef; parasites; nematodes; trematodes; ticks; prevention; treatment; parasiticide; acaricide; endectocide; economics of treatment

The global human population continues to increase at a rate of slightly more than 1% per year [1]. Meat consumption is an increasing source of nutrition for many people worldwide. As global population and income increase over the next decade, consumption of meat is expected to increase 14% by 2030 compared to average consumption during 2018–2020. Specifically, beef production is expected to grow 5.9% to supply a portion of this increasing demand for meat [2]. There are many challenges to profitable beef production, not the least of which is parasitism. Parasitic infections reduce liveweight, milk yield, feed efficiency and are a leading cause of liver condemnations, mortality in young cattle, and in some parts of the world, even mortality in adult animals [4–11]. Parasitic infestation can also negatively impact reproduction and immune response to vaccination and disease [12–18].

To maintain profitability, measures implemented to mitigate the impact of parasitism must result in a positive economic return on investment for cattle producers. Methods for successful parasite control change over time; parasite susceptibility to chemotherapeutics shift, new

management strategies are introduced, and parasite epidemiology fluctuates [3]. To assist beef producers and veterinarians, this paper aims to summarily review current trends on the impact from the most economically significant parasite species, across beef cattle production in temperate and subtropical areas, specifically considering return on investment.

2. Beef Cattle Parasitism: Estimating Losses in a Changing Landscape

The world's largest beef and veal producers are the United States of America (USA), Brazil, China, the European Union, Argentina, Mexico and Australia, which collectively contributed 74% of the estimated total 58,184,000 metric tons beef carcass weight produced in 2022 [19]. Losses due to parasitism in cattle are summarized in Table 2.

Table 2. Economic Burden of Parasites. Estimated Annual Losses from Parasites in Beef Cattle 1,2.

Country	GIN	Fluke	Tick	Internal Parasites and Ticks	References
Brazil	US$ 7.11 B	US$ 0.210 B	US$3.24 B	n/a	[20,21]
Mexico	US$ 0.45 B	US$ 0.13 B	US$ 0.57 B	n/a	[9]
Europe 3	€0.423 B (includes fluke)	n/a	n/a	n/a	[22]
Australia	AUS$0.0 936 B	n/a	AUS$ 0.161 B	AUS$0.2546 B	[23]
USA	US$ 8.5 B 4	US$0.00116 B 5	n/a	n/a	[19,24–27]

Cattle parasites causing the greatest economic losses in beef cattle production worldwide are nematodes, trematodes, and ticks. In addition to financial losses, parasitism impacts the health and welfare of farmed cattle. The World Organization for Animal Health defines good animal welfare when an animal is "… healthy, comfortable, well nourished, safe, is not suffering from unpleasant states such as pain, fear and distress, and is able to express behaviors that are important for its physical and mental state. Good animal welfare requires disease prevention and appropriate veterinary care, shelter, management, and nutrition. "[28]. Low level gastrointestinal nematode infection has been shown to negatively influence cattle feeding, rumination, resting and standing behaviors and thereby negatively impacting their overall welfare [29–32]. Tick infestations also impacts cattle welfare, not only in transmitting disease-causing organisms, but as ticks bite injure tissues at their feeding site, causing irritation, inflammation, hypersensitivity, and dermatitis [33] and can confer paralysis toxins [34].

Parasitism may also play a role in global meat production sustainability. Cattle and sheep infected with gastrointestinal nematode and liver fluke appear to contribute greater greenhouse gas emissions than uninfected animals [35-39]. With some governments issuing emission reduction targets, parasite control may become necessary to help satisfy increasing political and societal pressure for cattle producers to reduce their carbon footprint [40].

Successful, cost effective and profitable management of parasitism is dependent upon an understanding of the many variables that influence the level of infection. The types of parasites, the extent of their effects on cattle, and measures to control them differ by geography, climate, cattle genotype, age of cattle, production environment, and management practices. For example, tropical and subtropical environments are usually ideal for the life cycle of several parasites. As a result, the prevalence and variety of parasitic diseases in those areas are substantially greater than are those in temperate climates [347-51]. Consequently, parasite control methods developed for use in temperate areas will prove unsuccessful in other climates [50].

Cattle genotypes differ in their susceptibility to various parasite. For example, Bos indicus cattle tend to be more resistant to tick infestation than Bos taurus breeds [7,52-55]. Bos taurus breeds have been shown to be more resistant to GIN than Bos indicus breeds [56,57] although others could not substantiate this finding or have shown them to be more susceptible [53,55].

Young cattle, especially those on pasture for the first time, tend to have higher rates of GIN infection than adult cattle [57-59]. Even individuals within the same cattle class and management system will have varying degrees of inherent resistance to nematodes and ticks [60-63]. Cattle on pasture have greater exposure to nematodes and flukes than do cattle in feedlots [46,64-68]. Seasonality and nutritional status also affect cattle exposure and response to parasites [50,60,69].

Distribution of some parasites, and oftentimes the diseases they vector, is expanding because compatible environmental conditions that support their life cycle are expanding. Examples are the broader distribution of the tropical cattle tick (Rhipicephalus [Boophilus] microplus) in West Africa, and the recent (2017) discovery in the United States of the Asian long-horned tick, or bush tick, Haemaphysalis longicornis [70,71]. That tick is a vector for Theileria orientalis and could be a vector for parasite-borne pathogens in the USA [72].

While climate is an important driver of tick and helminth distribution, husbandry practices on the farm also impact parasite transmission to livestock [3,50,66,78,79]. Pasture management administered with knowledge of local parasite epidemiology can successfully limit cattle parasite exposure [3, 4, 43,46,66,80-82]. Limiting cattle exposure to the snail intermediate hosts by draining wet pastures or restricting access to such has been shown to reduce liver fluke infection [80,81]. Nutritional management can also reduce the severity of parasite infection or enhance immunity [49,84-86].

Worldwide, reports of parasite resistance to anthelmintics, endectocides and acaricides are increasing [79,87-95]. Anthelmintic resistance has even been identified in farms with no or low treatment history and without any epidemiological or trade links [99,103]. Control of liver fluke has long been challenging [15]. There are few drugs available to treat liver flukes and many of these have low efficacies against damaging juvenile stages of Fasciola hepatica. And now there is evidence of drug resistance in liver flukes [15]. Widespread parasiticide resistance will necessitate implementation of Integrated Pest Management (IPM), wherein the systematic combination of multiple pest control strategies is employed to achieve sustainable and profitable parasite control [82,107].

In addition to changes in parasite distribution and susceptibility, changes in consumer preferences are impacting beef production [3, 24, 108]. In some parts of the world, there has been a shift in consumer preferences towards “natural” or organically produced beef [108]. A portion of consumers are willing to pay a premium for these products.

Overall, changes in climate and its impact on parasite distribution, anthelmintic, endectocide and acaricide resistance, and consumer preferences will necessitate modifications in the way in which parasite control has been managed in the past [3,63].

3. Parasites of Economic Importance

Gastrointestinal and pulmonary nematodes, liver flukes (trematodes), ticks, flies, lice, and mites all cause economic losses and negatively impact animal health and welfare [3,9,10,20,22,77,114]. In addition, many of these pests serve as vectors for viral, protozoal and bacterial organisms that can cause devasting diseases such as anaplasmosis, babesiosis, East Coast fever caused by Theileria parva and heartwater caused by Ehrlichia ruminantium [44,115,116]. As shown above, cattle parasites causing the greatest economic losses in beef cattle production worldwide are nematodes, trematodes and ticks.

3.1. Nematodes—Major Species, Health Impact and Economics of Control

Nematode parasites are one of the most common and important limiting factors that affect the health and wellbeing of livestock [43]. Infection with nematodes results in decreased appetite, decreased gut retention time, and a net fluid, electrolyte, and nutrient loss to the gut lumen, which negatively impacts feed intake, growth rate, carcass weight, carcass composition, fertility, immune response and milk yield [43,117]. These production changes can be difficult to recognize but result in economic losses to the producer.

Studies comparing high and low efficacy anthelmintics indicate financial gains with high efficacy treatments [85,86] whereas it would be more effective not to treat than to use low-efficacy drugs [86]. Better cost effectiveness resulted from improved animal production indices when proper nutrition was combined with an anthelmintic treatment that effectively reduced nematode egg shedding [85].

Infection with the respiratory nematode, Dictyocaulus viviparus, or lungworm, can also negatively impact the health, welfare, and production efficiency of beef cattle. This parasite is found sporadically in pasture-raised cattle in Europe, North and South America, Brazil and Australia [117,121-123].

In Europe, 81% of the annual economic losses due to helminth infections, including GINs, lungworms and liver flukes, were due to lost production and 19% due to cost of treatment [22]. The use of broad spectrum anthelmintics has long been the go-to for the treatment of nematode infection in cattle [3]. With the introduction of truly broad spectrum anthelmintics, including the benzimidazoles, the pro-benzimidazoles, the imidazothiazoles and the tetra-hydro-pyrimidines in the 1960s, farmers achieved considerable success in the control of cattle nematodes [3]. The introduction of the macrocyclic lactones (ML) in the 1980s was a momentous improvement in nematode and ectoparasite control because the potency, convenience, spectrum, and eventual low cost of the MLs largely replaced the need for critical thinking about parasite control [89,113].

Anthelminthic resistance (AR) or lack of efficacy in nematodes of beef cattle are emerging issues globally with implications for effective parasite control. ML resistance results in significant production losses and treatment costs annually [22]. Across Europe, aggregated farm level prevalence of AR was 8% for benzimidazoles (BZ), 32% for MLs, 12% for levamisole (LEV) and 27% for moxidectin (MOX) [137].

AR makes reliance on the routine use of single class chemotherapeutics for effective helminth control no longer dependable. Treatment failure due to AR can have greater economic impact on cattle producers than no treatment because treatment costs are added to continued production losses [86]. Alternative strategies for effective and sustainable helminth control, designed to limit production losses due to nematode infection while maintaining the efficacy of available anthelmintics, have been investigated [138-142]. Two such strategies are targeted treatment (TT), and targeted selective treatment (TST).

Strategies that employ timely combination drug therapy have also been reported with success [67,93,143-146]. Walker et al. (2013) found administration of a benzimidazole with a macrocyclic lactone given at two different times provided GIN control and improved weight gains for stocker calves grazing warm-season pastures [143]. When resistance to avermectins was found in US pastured stocker cattle, a combination of eprinomectin and levamisole was found most effective against both intestinal and abomasal nematodes whereas levamisole treatment alone was not effective against Ostertagia ostertagi [98].

Knowledge of the unique AR landscape on individual farms will be necessary for devising economically sound deworming strategies [57,142]. Fecal monitoring can be a practical tool for parasite management on many farms.

Production and financial gain following nematode treatment has been demonstrated for all sectors of beef cattle production, in temperate, subtropical, and tropical climates [7,13,17,22,24,43,67,85,86,99,145,148-154].

3.2. Cattle Trematodes (Flukes)—Major Species, Health Impact and Economics of Control

Liver flukes, also known as trematodes, are found in more than 70 countries worldwide. Prevalence in cattle is variable, with ranges of 1.2–91% in Africa, 3–67% in the Americas (ex-USA), 1–69% in Asia, 26–81% in Australia/Oceania, and 0.12–86% in Europe [114]. The most encountered species is Fasciola hepatica, which is predominately found in temperate climates, but can also be found in tropical and subtropical countries, including those in the Middle East, South America and Asia. Within the host, immature flukes cause trauma and inflammation as they migrate through the liver, and upon entering the bile duct, they cause obstruction and cholangitis.

Fasciolosis causes huge financial losses to butchers, farmers, and consumers in the form of liver condemnation, poor quality carcass, reduction in growth rate and reduced productivity [114]. More often liver fluke infection causes subclinical disease with resultant reductions in feed efficiency, growth, fertility (delayed puberty and increased calving interval) and overall loss of productivity [45,64,68,156,159,160]. As few as 1 to 10 liver flukes have been found to increase slaughter age for animals [160].

Liver fluke control is only relevant for cattle raised in or purchased from areas where both liver fluke and the snail intermediate host reside. Liver fluke control programs must be customized according to local parasite transmission times, those times of year when pasture temperature and moisture are conducive to development of the snail and fluke, and incorporate an effective flukicide, efforts to reduce snail populations, and grazing management to limit liver fluke exposure [64].

Production gains following treatment for liver fluke have been well demonstrated [14,22,68,164-167]. In a review of 1582 published studies, Hayward et al. found treating flukes resulted in positive effects on daily weight gain, live weight and carcass weight of 9%, 6% and 0.6%, respectively. The authors also found that younger animals infected with flukes tended to have more severe effects on weight gain and that fluke infection tended to worsen live weight over time [68]. In a study of a commercial cow– calf operation in Louisiana, calves from cows receiving treatments for both flukes and nematodes had an average weight gain advantage of 8.9 kg in 205-day adjusted weaning weights compared with that of calves from cows receiving treatment for nematodes alone [158].

3.3. Cattle Ticks—Major Species, Health Impact and Economics of Control

About 80% of the world's cattle are affected by ticks and tick-borne diseases (TBD), both of which cause significant production losses [112]. Ticks of economic importance to cattle production occur worldwide and are broadly found in tropical and subtropical areas of the world [34,168,169]. Cattle in Asia, Australia and Central and South America are affected primarily with Rhipicephalus (Boophilus) microplus, whereas cattle across Africa are affected mainly by species from Rhipicephalus, Amblyomma and Hyalomma [115,170,171]. Ticks cause severe economic losses through the direct effect of tick attachment with the resultant "tick worry" and lost productivity, by injection of toxins and resultant "tick paralysis", by blood loss from tick feeding, and indirectly by their vectoring of disease-causing pathogens [34,42,61,172]. "

Production losses per tick and for various cattle breeds have been calculated [173-176]. For example, in a review of 19 papers, Jonsson et al. (2006) estimated daily production losses attributed to each R. microplus engorging female tick as approximately 1.37 g bodyweight in B. taurus cattle and 1.18 g bodyweight in B. taurus x B. indicus cattle. In a study of R. microplus control and its effect on beef cattle performance in the Brazilian Cerrado, Calvano et al. (2019) showed that tick infestation resulted in reduced weight loss equivalent to US$34.61 per animal in the backgrounding phase and US$7.97 per animal in the finishing phase for Brangus animals and crosses [172].

Ticks transmit a diverse array of pathogens including protozoa, bacteria, and viruses [11]. Tick-borne pathogens that affect cattle are among the diseases listed as notifiable by the World Organization for Animal Health. These include bovine babesiosis, anaplasmosis, theileriosis, and heartwater (Ehrlichia ruminantium) [11,78,177].

The cattle fever tick, R. microplus, is considered to be the most economically important ectoparasite of livestock worldwide [11, 34]. This invasive tick species is the vector of Babesia bovis and Babesia bigemina, causing babesiosis in cattle in tropical and subtropical parts of the world. Estimates place bovine babesiosis at the top of arthropod-borne diseases causing financial losses for cattle producers [11,34]. A meta-analysis of over 81,000 samples from 62 countries across six continents revealed an overall global prevalence of bovine babesiosis as 29%. Prevalence regionally was 52% in North America, 64% in South America, 61% in Australia, 22% in Europe, 27% in Africa, and 19% in Asia [178].

Other tick species also transmit TBD agents of economic importance. The agent responsible of bovine anaplasmosis is vectored by over 20 tick species, mainly Rhipicephalus spp. and Dermacentor spp., and is common throughout tropical and subtropical regions worldwide [179]. In Tanzania for example, economic losses from Rhipicephalus appendiculatus, which is the vector of a protozoan parasite, Theileria parva, causing theileriosis, also known as East Coast Fever, has a major impact on livestock farming in sub-Saharan Africa, killing over one million animals each year [6,180]. In one study, each engorging female tick was associated with a loss of 4 g body weight per day in B. taurus cattle [181].

There is evidence that ticks and TBD are increasing [178]. Climate change, with resultant movement of tick populations, and transportation of cattle from endemic to non-endemic areas have facilitated spread of TBD [179,182].

Routine, indiscriminate use of acaricides, and inaccurate dosing for tick control has contributed to acaricide resistance worldwide, including cross-resistance and multiple drug resistance (MDR), especially in R. microplus [82,95]. Resistance to most chemical classes, including organochlorines, organophosphates, carbamates, formamidines, pyrethroids and ML has been reported [79,83,95].

The occurrence of acaricide resistance requires more nuanced tick control measures. Alternative methods for tick control, including the use of naturally tick resistant cattle, biological control (biopesticides), grazing management for tick population control, and tick vaccines, have also been employed with variable success [61,112,172,182,186,187]. A combination of various tick control methods, individualized for each farm, will provide the best protection against ticks and TBD in cattle [182].

4. Implementing Parasite Control Measures

Emergence of anthelmintic and acaricidal resistance beg the question, can parasiticides be used effectively and economically? With changing sensitivity of parasites to anthelmintics, endectocides and acaricides, cost effective treatments must be devised that provide positive return on investment considering all input costs. A threshold of parasitism above which treatment outcome results in

positive return on investment, but below which non-treatment is the more prudent response must be determined. For example, with mild infection, cattle may not show any measurable adverse effects. Above a certain threshold of infection economic impact due to reduced production results.

5. Parasite Control by Production Type

Selection and use of anthelmintics and acaricides based on parasite prevalence, resistance profile, along with appropriate timing of administration, is key to successful implementation. Fecal egg count monitoring and nematode identification via PCR can be valuable for guiding chemotherapy selection [144]. For tick control, suspected acaricide resistance should be confirmed in the laboratory, when possible, with assays such as larval packet test (LPT) for example [79,82,83]. Cattle producers in each production sector, cow-calf, stocker/pasture raised, and feedlot can adopt sector appropriate parasite control strategies to enhance cattle health, performance and profitability [24].

5.1. Cow-Calf

Irrespective of geography, all grazing cattle, including cow-calf herds, are exposed to gastrointestinal nematodes (GIN) that contribute to productivity loss [43]. For cow-calf operations productivity is measured by pregnancy rate, average daily gain (ADG) and calf weaning weight [24]. Treatment to control GIN in heifers and cows has been associated with productivity gains in cow-calf herds and replacement heifers [14,18,152,191,192]. In a review of 170 research studies to determine the economic benefit of various pharmaceutical technologies including parasite control, growth promoting implants, sub-therapeutic antibiotics, ionophores, and beta agonists, based on US pricing in 2005, Lawrence et al. (2007) found that 73% of cow-calf operators utilize de-wormers and 81% utilize fly control. Their analysis found that parasite control in the cowherd has a significant positive impact on calf production with de-wormers increasing weaning rate of 23.6% [24]. For example, Stromberg et al. (1997) showed, over a 2-year study, that cows treated with fenbendazole at spring turnout and retreated along with their calves midsummer had calves that significantly outgained the control calves in both years. Average daily gain (ADG) for calves and reproductive performance for cows were both significantly greater than for control cattle. The pregnancy rate averaged across both years was 94% for the treated cows compared to 82% for the control animals [13]. While return on investment was not calculated in this study, the advantage of the ADG, the value of kilograms of calf-weight sold, the number of days on pasture, the cost of the anthelmintic product, and an appropriate cattle handling charge can be used to calculate the return. The results also indicated significantly reduced parasite egg shedding and pasture contamination.

Strategic use of anthelmintics can also be used to influence reproductive performance in estrus synchronized cows [17,152]. The economic efficiency of parasite control over long versus short-term periods (PC-LT vs. PC-ST) on reproductive performance of estrus synchronized Angus cross beef cows in North America was studied by Johnson et al. (2020). The authors found long-term parasite control effectively reduced parasite load, maintained or gained body condition and contributed to improved pregnancy outcomes. [17]. Similarly, in Brazil the use of anthelmintics with estrus synchronization and timed artificial insemination (TAI) was shown to increase pregnancy rate following the first TAI and elicit positive ROI in Nelore cows on pasture naturally infected with GIN [152].

Where exposure to liver fluke is high, adding a flukicide to nematode control has been shown to produce higher condition scores and weight gains than when heifers were treated for nematodes alone [14]. In temperate regions, recommended production-based treatment threshold for liver fluke

is an individual FEC greater than 5 eggs per gram of feces (EPG) and for herds with greater than 25 percent prevalence [65].

5.2. Weanling-Stocker-Pasture Cattle

Stocker cattle and those finished on pasture are routinely exposed to GIN. Therefore, it is not surprising that of the available production enhancing pharmaceuticals including parasite control, growth promoting implants, sub-therapeutic antibiotics, ionophores, and beta agonist, de-wormers affect average daily gain (ADG) the most in stocker operations [24]. Numerus studies have shown that anthelmintics used in first and second season grazing cattle contribute to increased weight gain ranging from 11.85 to 49 kg per animal, when compared to non-dosed animals [99,149,193,194]. Knowledge of regional nematode prevalence and anthelmintic efficacy is critical for successful outcome and positive ROI. For example, no advantages in average daily gain in grazing cattle treated once with 3.5% doramectin, 3.15% ivermectin, or 1% doramectin compared to non-treated cattle resulted when nematodes were resistance to avermectins [99].

Since nematode species such as Haemonchus spp. are more pathogenic than Cooperia spp., identity of both parasite species present and resistance profile is needed. Strategies such as targeted treatment or targeted selective treatment may help slow AR [140,141,194-198]. Increased costs associated with the testing necessary to implement TST must be considered [140]. To be effective each farm will need to consider local practices, epidemiological, and economic factors to devise the optimal treatment strategy for a specific farm [139].

Timing of anthelmintic use is also critical for optimizing GIN control and ROI. Studies in the USA have shown that three anthelmintic treatments administered in May, August and November were more effective for weight gain than three treatments in May, July and September and more effective than two treatments administered in May and November [145,155]. Proper nutrition and anthelmintic selection can also impact ROI.

Nematode control in grazed stocker cattle benefits cattle at the feedlot as well. Cattle effectively treated for GIN prior to feedlot arrival, delivered numerically greater total income per steer than did cattle arriving with high GIN egg counts despite deworming on arrival at the feedlot [67,150,199]. Steers dewormed at the feedlot did respond to anthelmintic intervention, but they did not experience compensatory gain during the feedlot phase and tended to have altered carcass composition and reduced marbling scores at slaughter [150]. Yazwinski et al. (2015) also found fewer illnesses during time in the feed yard for feedlot cattle that were treated for GIN while on pasture prior to arrival at the feedlot [67]. These studies show that GIN control in growing cattle might be economical for both grazer and feedlot operator and provides further justification for implementation.

Where ticks are prevalent, adding tick control to nematode control contributes to production gains and financial returns [7]. Bianchin et al. (2007) showed treatment of B. taurus x B. indicus steers in subtropical region of Brazil Cerado with three anthelmintic treatments, alternating albendazole and doramectin or doramectin alone, during the winter, and three fipronil insecticidal-acaricidal treatments during the spring/summer provided significant additional weight gain. Steers treated for GIN gained a mean of 33 kg more than untreated steers. Steers additionally treated for ectoparasites had additional mean weight gains of 13 kg compared with non-treated steers [7].

In pastured and silage supplemented, 8- to 9-month-old B. taurus x B. indicus cattle co-parasitized with R. microplus, H. irritans, and GINs, in subtropical southeastern Brazil, Gomes et al. (2022) found the treatment protocol utilizing an ectoparasiticide plus an endoparasiticide showed better outcomes regarding parasite counts, productivity, and financial data than strategic treatment

using an endectocide alone [153]. Studies showed, while strategic treatment with endectocides is popular in Brazilian cattle production for its convenience, financial returns from treatment with an ectoparasiticide along with an endoparasiticide can deliver better ROI.

5.3. Feedlot

Parasiticides, avermectins and fly control are the most commonly used pharmaceuticals in feedlots in the United States [24]. Cattle destined for the feedlot invariably come from pastures where they are exposed to GIN, therefore cattle are oftentimes treated for these parasites upon feedlot arrival. Since nematode transmission is not a problem in the feed yard, short acting anthelmintics such as oral fenbendazole have been shown to improve weight gain and carcass grade with significant financial returns [154]. MLs, combined with a benzimidazole or imidazothiazole, are also commonly used [67]. Adding a benzimidazole or imidazothiazole along with the ML removes nematodes resistant to the ML while the ML removes arrested nematodes, such as O. ostertagi, and controls lice, flies and ticks. With this treatment, production parameters (ADG, feed efficiency [FE], and dry matter intake [DMI]) have been shown to improve for cattle whether they were previously treated or not. However, improvements in ADG, FE, and DMI were greater in stocker cattle previously untreated, with increases of 13.4%, 4.9%, and 7.1%, respectively, wherein stocker cattle that were previously treated while on pasture had improvements of 4.2%, 0.3%, and 2.8%, respectively [67].

In the feedlot, cattle infected with liver fluke have decreased ADG and FE, poorer carcass quality scores, and bring lower prices gains compared to uninfected cattle [156-158,199]. However, by the time cattle previously exposed to liver fluke arrive at the feedlot, damage from liver fluke infection has already been inflicted, therefore, treatment with flukicide at this stage is oftentimes futile [67,200].

9. Conclusions

Parasitism in beef cattle negatively impacts liveweight, feed efficiency, reproduction, calf yield, carcass quality, is a leading cause of liver condemnations and may be implicated in the increase production of greenhouse gasses. These production losses can be significant and negatively impact the quantity of meat produced necessary to feed an increasing population. Losses increase the cost of meat production and result in diminished financial returns for beef producers while contributing to alterations in the atmospheric environment and climate. However, implementing strategic parasite control measures, with thorough knowledge of parasite risk, prevalence, parasiticide resistance profiles, and prices can result in positive economic returns for beef cattle farmers in all sectors and partially address concerns associated with the agricultural production of greenhouse gases.

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Cryptosporidiosis in cattle: recent advances in prevention

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Cryptosporidiosis is a relevant component of neonatal calf diarrhea syndrome

Neonatal calf diarrhoea (NCD) is one of the most relevant causes of mortality and morbidity for young dairy or beef calves (Murray et al., 2016, Tora et al., 2021; NAHMS-USDA, 2021). Some pathogens causing NCD are zoonotic (Holland, 1990). Additionally, NCD is a significant factor contributing to the use of antibiotics on many dairy and beef farms, primarily for treating young calves with diarrhoea (Eibl et al., 2021; NAHMS-USDA 2021).

The most common worldwide diarrhoea-causing agents in neonatal calves are *Cryptosporidium parvum*, bovine rotavirus (BRV), bovine coronavirus (BCoV), and enterotoxigenic *Escherichia coli* F5 (ETEC) (Conrady et al., 2021, Holland, 1990). Cryptosporidiosis is now recognised as endemic in cattle worldwide. Several studies have confirmed that globally this protist is the most prevalent infectious agent diagnosed in cases of neonatal enteritis in calves (Conrady et al., 2021). Some paradigmatic examples confirm this trend in different geographical regions and time periods. In Spain, (i) *Cryptosporidium* spp. and BRV were the most detected agents (52.3% and 42.7%, respectively) in faecal samples collected from diarrheic dairy calves; (ii) ETEC and BCoV were detected in 11.9% and 7.3% of the samples, respectively; (iii) *Salmonella* spp. was only found in the faeces of two calves (0.9%); (iv) mixed infections with two or more agents occurred in 28% of the calves; and (v) concurrent infections of BRV and *Cryptosporidium* spp. were found in 21.6% of the calves (de la Fuente, 1998). Veterinary surveillance reports in the UK between 2007 and 2011 showed that cryptosporidiosis was the main diagnosed cause of enteritis in calves (57% alone or in mixed infections) (VIDA report, 2014). In China, the most detected pathogens in cases of calf diarrhoea were *Cryptosporidium* spp. (50.46%), BRV (23.18%), ETEC (20.00%), and BCoV (11.82%). Other pathogens such as coccidia (6.90%), bovine astrovirus (5.46%), bovine torovirus (4.09%), and bovine kobuvirus (3.18%) primarily existed in the form of mixed infections (Wang et al., 2023). Some of these viruses have been detected in diarrheic calves. In fact, it has been suggested that immunosuppressive viruses may predispose animals to *C. parvum* infection. However, their significance as primary pathogens remains to be elucidated (Cho et al., 2013).

Four *Cryptosporidium* species are regularly detected in cattle, namely *C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni*. The prevalence of these species is age-related. *Cryptosporidium parvum* is mainly responsible for infection in suckling calves (under three weeks of age) and associated to NCD (Thomson et al., 2017; Hoque et al., 2023). *Cryptosporidium bovis* and *C. ryanae* are mainly identified in post-weaned calves with no signs of clinical disease. *Cryptosporidium andersoni* can be detected in the abomasum of juvenile and adult cattle and seems to exert an influence on milk production with no further clinical signs. Occasionally, *C. bovis*, *C. ryanae* and *C. andersoni* have been reported in pre-weaned calves (Thomson et al., 2017). In a recent study carried out with healthy cattle from the North-West of Spain, the prevalence of *Cryptosporidium* spp. was overall high, but the zoonotic *C. parvum* was the most frequently identified species in all age classes, which poses significant animal and public health concerns. Although healthy calves under 1 month were the major carriers of *C.*

parvum, adult cattle may also play a role in the occurrence of cryptosporidiosis outbreaks in calves and humans (Diaz et al., 2021).

A recent systematic review and meta-analysis in dairy calves estimated a worldwide *C. parvum* infection prevalence of 21.9% (7755/42,890; 95% confidence interval: 19.9–23.9%) from 2000 to 2021.

Cryptosporidiosis is spread by faecal-oral transmission, either directly via contact with faeces from infected hosts, or indirectly through environmental contamination or ingestion of contaminated food or water (Adkins, 2022; Thomson et al., 2017).

The life cycle of *Cryptosporidium* spp. is direct and develops in the lower digestive tract of cattle after ingestion of the environmental resistant form: the oocyst. The parasite multiplies in the enterocytes following a merogonic cycle with two generations of meronts, a gametogonic cycle with macrogametes, microgametes, and zygotes, and a sporogony (de Graaf et al., 1999). Particularities of the cycle with biological and pathological implications are the following (summarized from Current & Garcia, 1991; de Graaf et al., 1999; Thomson et al., 2017): (i) the infective dose to initiate the infection is as low as 17 oocysts (Zambrinski et al., 2013). (ii) The parasite develops inside the epithelial cell of the digestive tract, although on the edge of the host cell cytoplasm and separated from it by a feeder organelle membrane; this intracellular extra cytoplasmatic location is unique and plays a major role in the failure of many antimicrobial agents to inhibit the growth of *Cryptosporidium*. (iii) Two stages can cause autoinfection: the recycling type I meronts and the thin-walled oocysts; consequently, in the absence of a protective immune response, *Cryptosporidium* may persist inside a single host, even without further exposure to exogenous oocysts. (iv) The thick-walled oocysts are fully sporulated and infectious when they leave the body within the faeces. (v) Infected calves shed large number of oocysts (>10^{10}/ day); these are extremely resistant to disinfectants, can travel a considerable distance following runoff, and can survive for a relatively long time in an aqueous environment. In fact, the total global *Cryptosporidium* spp. load from livestock manure has been estimated in 3.2×10^{23} oocysts per year, being calves the main contributors (Vermeulen et al., 2017). (vi) Different mammal species, including domestic ruminants and humans, are suitable hosts for the parasite. As a result, *C. parvum* has an exceptional capacity to disseminate and numerous infection sources (Current and Garcia, 1991, de Graaf et al., 1999; Thomson et al., 2017; Adkins, 2022).

Transmission of *C. parvum* infection to calves still has some major knowledge gaps, such as the initial source of oocysts, and how these oocysts can persist between calving periods (Shaw et al., 2021). Some studies have examined the role of adult cattle in the transmission of *C. parvum* oocysts (Atwill et al., 1998; Faubert & Litvinsky, 2000). Using a highly sensitive faecal detection methodology to genotype faecal samples, a recent work aimed to determine if adult cattle could be a source of *C. parvum* infection for their calves. The results showed that the calves within each herd had a predominant single multilocus genotype, whereas adult cattle had multiple distinct genotypes. Interestingly, adult cattle from dairy farms tested before calving had a multilocus genotype that was different from that detected in their calves in most of the cases. In contrast, the multilocus genotype of adults and calves from beef farms matched, although faecal samples were taken at the same time (Shaw et al., 2021).

In addition to the parasite factors described above, little information is available on differences in infectivity, excretion patterns, virulence, or immunogenicity among different isolates of *C. parvum*

from cattle or other sources. However, several environmental and management factors have been associated to the presence of *C. parvum* infection. Seasonality was extensively reviewed by Adkins (2022) and found to be a significant factor. Herd size is also relevant, with large operations having a higher number of calves testing positive for *Cryptosporidium* spp. compared to medium and small farms. Separating pregnant cows from the rest of the herd before calving can be protective, likely because carrier adults shed oocysts inadvertently. Concrete flooring in calf housing reduces the risk of cryptosporidiosis, while earth or gravel flooring increases it. Finally, hygienic measures have a protective effect on the prevalence of *C. parvum* infection (Adkins, 2022; de Graaf et al., 1999; Harp & Goff, 1998; Urie et al., 2018).

Economic and public health impact

The economic impact of cryptosporidiosis derives from: (i) the high morbidity rates; (ii) the increased mortality rates; (iii) the exacerbated disease in coinfections with other enteropathogens; (iv) the treatment cost of sick animals; and (v) the reduced animal performance due to a retarded growth (estimated at £128 (~150€)/affected calf) (Shaw et al., 2020). However, studies investigating the long-term effects of *C. parvum* infection in calves remain to be conducted (Adkins, 2022). In some countries as the UK, relationship between livestock and human infections has been demonstrated, consolidating the role of cattle as primary reservoirs of zoonotic cryptosporidiosis (Thomson et al., 2017).

Infection dynamics, clinical signs and lesions

The main clinical presentation of *C. parvum* infection in calves is diarrhoea. Other clinical signs include depression, dehydration, decreased feed intake, abdominal pain, and tenesmus. The severity and duration of disease are highly variable. Several factors are involved in the severity of clinical disease, including immune status, nutritional status, virulence of the pathogen, and the occurrence of coinfections (Anderson, 1998).

Prepatent and patent periods range from 3±6 and 4±13 days, respectively (Anderson, 1998). Oocyst excretion can start as early as 3 days of age. A great variability is observed in the severity and duration of diarrhoea due to cryptosporidiosis, even when the animals were exposed to similar conditions. In most calves, diarrhoea has already begun at 3±5 days post-infection (dpi), and lasted from 4 to 17 dpi (Anderson 1981). The period of maximum risk is the second week of life. It has been described that that the earlier the animals acquired the infection, the longer the patent period. Oocyst shedding, which does not always begin with the onset of diarrhoea, can last between 8 and 23 days (mean 12.4 ± 3.3 days (Castro-Hermida et al., 2002). Cryptosporidial diarrhoea is associated with the excretion of tremendous numbers of oocysts. While *Cryptosporidium* infection is mainly concentrated in the distal small intestine, lesions can also be found in the caecum and colon, and occasionally in the duodenum. The pathological findings associated with *Cryptosporidium* infection are a mild to moderate villous atrophy, villous fusion, and changes in the surface epithelium (reviewed in de Graaf et al., 1999). Coinfections with other enteric pathogens have been documented to contribute to the severity of cryptosporidiosis. Calves with severe cases can take 4 to 6 weeks to fully recover. However, mortality is generally low (Adkins, 2022).

Recent microbiome studies performed in *C. parvum*-infected calves have shown a shift in microbial communities. This dysbiosis features a higher occurrence of virulence genes attributed to exotoxins,

adherence factors, and secretion systems from *Clostridium* spp. and other enteropathogens, including *Campylobacter* spp., *Escherichia* sp. *Shigella* spp., and *Listeria* spp. (Gamsjäger et al., 2023). Moreover, *C. parvum* infection is associated with an increase of *Fusobacterium* spp. (Dorbek-Kolin et al., 2022), and a negative impact on bacterial diversity.

Importantly, disruption of the microbiome through factors like inflammation, dietary changes, or antibiotic use has been shown to increase the risk of cryptosporidiosis.

Control measures

Control of cryptosporidiosis is required to diminish economic losses derived from neonatal diarrhea in newborn cattle and to interrupt its transmission to humans and animals through water, food and contact with infected animals (Yoder and Beach 2010). Accordingly, the control of bovine cryptosporidiosis should tackle (i) a significant amelioration of the disease burden in livestock, and (ii) a drastic reduction of the oocyst shedding in newborn ruminants. At present, control pillars rely on and adequate management and hygiene measures during peripartum and neonatal periods in dams and their offspring. This should be complemented with supportive therapy and therapeutic treatment of diseased newborns. Recently, a new commercial vaccine has been registered in the EU for their use in cattle (Bovilis Cryptium®). The most relevant issues are summarized below.

Use correct biosafety measures and avoid risk factors associated with calf management practices. The control measures must consider basic biosafety measures aimed at preventing faecal-oral transmission of oocysts. This should consider the biological characteristics of the parasite discussed above, as well as the management and zootechnical factors that may pose a risk factor for transmission. The objective is to reduce the early exposure of calves to *C. parvum* infection.

a. Sick calves should be housed apart from other calves.
b. Caretakers should always take care of healthy calves before treating or handling sick calves. A separate set of boots and coveralls should be used by caretakers when caring for sick calves.
c. Thorough cleaning of stalls before introducing new calves is crucial whenever possible, depending on the farm's setup. While most commercial disinfectants at recommended concentrations fail to neutralize *C. parvum* oocysts, the parasite is vulnerable to extremes in temperature and desiccation. Therefore, cleaning with hot water followed by complete drying is an effective method for eliminating *C. parvum* oocysts.
d. Risk of zoonotic transmission, especially to small children or the elderly, who may be most susceptible to disease, should always be considered.

Colostrum transfer. Implementing good management practices can significantly strengthen calves' resistance against other enteric pathogens. These practices include ensuring timely access to high-quality colostrum to prevent passive transfer failure and providing young calves with warm and dry environments.

Use of supportive therapy to treat the diarrhoea clinical signs. Calf diarrhea is typically treated without targeting specific pathogens, as treatment approaches often overlap. While cryptosporidiosis in neonatal calves usually resolves on its own, supportive care is crucial. This includes replacing lost fluids and electrolytes, restoring acid-base balance, and potentially

using anti-inflammatories. Detailed recommendations for managing undifferentiated calf diarrhea have been reviewed earlier (Constable 2009; Smith & Bertchold, 2014).

Chemotherapeutic treatment. Concerning therapeutic tools, halofuginone lactate is the only marketed drug available to treat sick calves in several European countries. The mechanism of action of this drug is unknown, but it is thought to affect the merozoite and sporozoite stage of the parasite (Naciri et al., 1993). This drug is approved for use in both prevention and treatment of cryptosporidiosis in calves, although in the EU cannot be used as a prophylactic tool. As a prophylactic measure the drug should be given within 48 h of birth, and as a therapeutic agent, within 24 h of the onset of clinical signs. Paromomycin sulfate is also marketed in a few countries to treat calves. However, both drugs only induce a partial reduction in oocysts shedding and in the severity of the clinical signs. Moreover, these drugs cannot be used in animals with signs of diarrhoea for more than 24 h, and need to be administered for a few consecutive days which can be difficult to manage. In addition, they have other safety drawbacks (for more details see table 1). Hence, more effective strategies are urgently required. In this regard, a high number of therapeutic agents has been tested against cryptosporidiosis in different experimental models but with limited success. Among this, decoquinate, that is marketed to treat coccidiosis, had no effect on the levels of diarrhea or dehydration, the proportions of diarrheic calves, or the proportions of calves shedding oocysts (Lallemond et al., 2006). In contrast, a bumped kinase inhibitor (BKI) targeting calcium-dependent protein kinase 1 (CDPK1) showed promising efficacy in a *C. parvum* neonatal calf model, reducing faecal oocyst excretion and promoting weight gain (Van Voorhis et al., 2021). This proof of concept provides evidence that BKI-based therapy could offer a highly effective option for treating cryptosporidiosis in the future.

Table 1: Marketed drugs to treat cryptosporidiosis in calves.

	Halofuginone lactate	Paromomycin sulfate
Family	Quinazolinone derivatives group	Aminoglicoside antibiotic
Spectrum	Antiprotozoal effect (active against free parasite stages: sporozoite, merozoite)	Broad spectrum with bactericidal and antiprotozoal effect.
Marketed against bovine cryptosporidiosis in Europe	+	+ (only in a few countries)
Posology	Oral administration, 7 consecutive days (100-120 µg/Kg bw/d)	Oral administration, 5 consecutive days (150mg/Kg bw/d)
Additional therapeutic usage	No	Treatment against gastrointestinal disease by susceptible E. coli strains to paromomycin
Effect on intestinal microbiota in calves	Expected to be low	High
Toxicity	Narrow safety index Overdosing: diarrhea, blood in faeces, anorexia, dehydration, prostration and abomasitis.	Antimicrobial resistances, ototoxicity, nephrotoxicity
Withdrawal period	Meat: 13 d	Meat: 110 d

The recent advances done in alternative therapies show limitations and lack of conclusive field results. Some plant extracts and yeast fermentation products have been tested but despite the promising results obtained they did not offer higher efficacy than commercial drugs.

Prebiotics and probiotics. Prebiotics and probiotics have emerged as a potential alternative to antibiotics for (i) promoting intestinal health, and (ii) reducing the incidence of diarrhoea. Indeed, probiotic supplementation is a frequent management strategy in dairy heifer operations (Branco Lopes et al., 2023).The goal is to restore a healthy gut microbiota and alleviate diarrhoea in calves. The rationale under this strategy relies on the knowledge about the microbiota colonization and the immunomodulation exerted in the gut of the newborn ruminants (Du et al., 2023). Despite the extensive research conducted on probiotics supplementation in dairy calves worldwide for more than four decades, the significant variability among studies in terms of experimental design underscores the need for standardized guidelines in clinical trials (Branco-Lopes et al., 2023). Notably, in most studies the cause of diarrhoea was often undetermined, the sample size was not well-justified, and the data was not adequately reported. Thus, there is a lack of specific controlled trials to assess the efficacy of probiotics against cryptosporidiosis. Two recent trials assessing the effectiveness of probiotics in preweaned dairy calves yielded divergent results: one showed no effect against *C. parvum* infection (use of fermented milk with *Bifidobacterium animalis* subsp. *lactis*, subsp. *Bulgaris, Streptococcus thermophilus* and *Bifidobacterium longum* subsp. *Longum)* (Fresno et al., 2023), while another (use of *Bacillus subtilis*) demonstrated only a reduction in oocyst shedding without affecting the duration of diarrhoea (Lucey et al., 2021). These discrepancies may be influenced by factors such as the posology, dose, and probiotics composition. In contrast, in the same study the use of a prebiotic (yeast culture enriched with mannan-oligosaccharide) did not yield a significant effect.

Vaccination. Infection is more common and severe in calves due to the immaturity of their adaptative immune responses. The intestinal epithelium seems to be important in the response against the parasite, as *Cryptosporidium* does not invade deeper tissues. The innate immune response is key, as infection leads to the transcription of inflammatory mediators and the secretion of antimicrobial peptides, a response primarily mediated by TLR2 and TLR4.Thanks to a strong cell-mediated response, calves infected with *Cryptosporidium* clear the infection after 2 weeks and become resistant to a second challenge with the same isolate. During a primary infection there is a marked increase in the number of CD4+, CD8+ and γ/δ T cells and the expression of pro-inflammatory cytokines in the affected tissues. After a secondary infection, there is a substantial increase in the number of CD4+ T cells, and a marked migration of CD8+ T cells to the epithelium of the intestinal villi, where the parasite is located (Abrahamsen 1998; Crawford & Kol, 2021; Gookin et al., 2002).

The most feasible strategy accepted for an effective immunization is the parenteral vaccination of pregnant heifers and cows to confer passive immunity to their calves via colostrum, similarly to what occurs for vaccination against other enteropathogens (rotavirus, coronavirus, and *E. coli* K99). There is experimental evidence supporting the benefit of passive transfer of antibodies developed against recombinant gp40 (cattle), CP15 (cattle, sheep, and goat) and P23 (cattle) glycoproteins to ameliorate cryptosporidiosis. Protein glycosilation is one of the major post translational modifications in *Cryptosporidium* and has a key role in parasite adhesion/attachment to host cells, and stimulation/evasion of the host immune responses. In addition, gp40, CP15 and P23 are present in the sporozoite and merozoite stages and potentially involved in the initial phases of the infection. Vaccination with these antigens resulted in the generation of specific antibodies in the hyperimmune colostrum, whose intake resulted in a significant reduction in oocyst shedding, and the duration and severity of diarrhoea in the offspring born from immunized dams (Askari et al., 2016; Bhalchandra et al., 2023; Sagodira et al., 1999). Nevertheless, the efficacy of such vaccines has yet to be demonstrated in the field and the role of cellular mediated immune response should be also a matter of further research. A new commercial vaccine is registered in the EU for their use in

cattle (Bovilis Cryptium®). This formulation is based on the use of arizidine treated-gp40 glycoprotein, that is produced in insect cells and formulated with oil (ISA 70) plus aluminum adjuvant. The results of a controlled trial showed that the vaccine is safe, can induce high anti-gp40 antibody titres, and reduces the average score diarrhoea in the treated calves. These promising findings guarantees further investigation under field conditions to assess their effectiveness.

Conclusions

The development of both new vaccine formulations and safe and effective drugs, together with the improvement of biosafety and correct management of the newborn calf, are opening very promising avenues to effectively control NCD caused by *C. parvum*. This approach holds significance not only for its economic relevance, but also for its impact on Public Health from a One Health perspective. Looking ahead, advancements in the knowledge of the intestinal microbiome and its modifications during disease, as well as a better understanding of the local immune response, could further illuminate the intricate interplay between this zoonotic pathogen and its environment, paving the way for targeted control strategies.

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Pharmacology and toxicology

Recrystallization of enrofloxacin to generate a solvate with outstanding physicochemical and pharmacological properties for bovine medicine.

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Aim

It is known that modifying the physicochemical properties of drugs can transform old molecules into new active principles. One approach to achieve this is synthesizing a crystal polymorph of the parent compound. Considering the noticeable potency, wide-spectrum, reasonable stability, and outstanding clinical success of enrofloxacin, it was decided that the rather water-insoluble parent compound could be recrystallized and turn into a polymorphic crystal/solvate to favor hydro-solubility and modify/improve its pharmacodynamic and pharmacokinetic properties. Thus, through a patented reaction (Patent 472715: Instituto Mexicano de la Protección Industrial, Mexico), a new enrofloxacin hydrochloride-dihydrate entity named enro-C was developed. The structure of the new solvate/crystal was defined by high-resolution mass spectrometry, Fourier-transformed infrared spectroscopy, X-ray powder diffraction, and thermal analysis. The water solubility studies of enro-C show a 20-fold increase compared to the parent enrofloxacin. An outstanding bioavailability of enro-C has been observed in various studies in cattle (1, 2). Enro-C has a pH ranging from 5.8 to 6.4 in water but can be prepared for neutrality. Considering the above, it was considered consequential to take advantage of these physicochemical features of enro-C in the following clinical scenarios: (1) as intramammary (IMa) preparation to treat either non-complicated mastitis or (2) chronic cases of mastitis, enhancing its antibacterial effects with anti-biofilm molecules and in a ten day IMa treatment period for enro-C-dimethyl sulphoxide (DMSO)-chitosan vs. IMa administration of ceftiofur; (3) enro-C was also incorporated into a hydrogel to handle newborn calves' navels; (4) based on lack of irritating or toxic effects obtained in in-vitro cell-culture studies, enro-C was tested as intrauterine antibacterial in 28 days postpartum, endometritis-affected dairy cows; and (5) finally, an efficacy study challenging enro-C vs the reference enrofloxacin-LA was carried out in calves affected by severe cases of bovine respiratory complex.

Material and Methods

In all the referred studies, the experimental setting followed the following standard features: they were all field clinical cases of the different diseases or conditions, i.e., none of the diseases was induced; a reference or gold-standard treatment was included in all listed trials, and no untreated bovines were ever included as participant bovines belonged to various commercial farms. In all studies, the following parameters were evaluated: post hoc GPower® test to appraise the power of each test in its clinical setting. Also, chi^2 and repeated measurement tests with a statistical significance of $p < 0.05$ were carried out to assess the clinical significance of the data. When possible, the bacteriological efficacy of the treated cases (enro-C and reference treatment) was studied. The

statistical significance of the effect of enro-C preparation on the causative etiological agent was also determined in some studies, i.e., chronic mastitis.

Results

As intramammary (IMa) preparation to treat non-complicated mastitis, enro-C has shown in (1) As intramammary (IMa) preparation to treat non-complicated mastitis, enro-C has shown in a total of 546 cows in 8 dairies a clinical success rate higher than 95%. It is statistically higher than the clinical efficacy achieved with the IMa administration of ceftiofur (68%) ($p < 0.05$). The probability of bacteriological cure was higher in the enro-C treated cows but not at a statistically significant level when compared with IMa ceftiofur ($p > 0.05$); (2) Results in this phase demonstrate (in 20 cows per group) that the experimental preparation of enro-C/DMSO/chitosan possesses the capacity to inhibit the formation of biofilm both in vitro and in vivo and degrade mature biofilm and has a well-defined microbicidal effect on both planktonic bacteria, as well as those contained inside the biofilm. A 100% and 80% efficacy were recorded for clinical and bacteriological cures in the affected cows, respectively. These values were statistically higher than the ones obtained with the IMa administration of ceftiofur ($p < 0.05$), i.e., 60 and 50 percent for clinical and bacteriological cure, respectively; (3) A trial was conducted with 414 newborn calves randomly divided by risk blocks of developing omphalitis or omphalophlebitis: low, medium, and high. The treatments [enro-C hydrogel ($\eta = 209$) or iodine-polyvinylpyrrolidone (I-PVP; $\eta = 205$)] were applied by stump-dipping daily for three days. There was a statistically significant difference in favor of the enro-C hydrogel ($p < 0.01$). The umbilical stump involution was evident in the enro-C-treated animals from day one, when most stumps became noticeably drier. Umbilical stump detachment occurred within a maximum of 28 days (25.5 days) in the enro-C/hydrogel-treated animals, and no umbilical scar presented infection. These results contrast with the I-PVP treated calves in which the umbilical stump detachment required an average of 10 more days, and 12 cases of omphaloflebitis, and two fatalities, were recorded; (4) In 50 cows (25 per treatment, randomly assigned, and all treated twice with closprostenol) with endometritis grade 3 to 5 (of 5 possible severity grades), and at 24 days postpartum, it was shown that the intrauterine (IU) administration of 500 mg/enro-C (one every 24 hours) in aqueous suspension, was statistically superior ($p < 0.05$) to the intrauterine administration of 500 mg of cephapirin, administered in the same manner, as far as the number of cows in which vaginal discharge disappeared; the number of days needed for the vaginal discharge disappearance; and number of days from partum to the first heat. The following variables were better in the enro-C treated cows but were only statistically different at $p < 0.1$: conception rate and number of inseminations to conception; (5) The treatment of 30 calves (15 calves per treatment) affected by a severe form of bovine respiratory complex graded 3, from 4 severity levels, was clinically more effective in calves treated with enro-C (10 mg/kg/day) as compared to the injection of the enrofloxacin-LA regarded as referent in this study (10 mg/kg every three days) ($p < 0.05$).

Conclusions

Derived from the tests carried out, it becomes evident that enro-C can be regarded as an excellent alternative to treat the included diseases in this report. It is proposed that the increased water solubility of enro-C, its format as a new solvate, and its recrystallization have given rise to a new pharmacological entity with unique features. For example, enro-C can also show outstanding efficacies in treating standard-uncomplicated mastitis and chronic cases. It is effective for treating pneumonia more effectively than the long-acting preparation of the reference enrofloxacin. When prepared as hydrogel, it resulted to be a preventive preparation for the development of omphalophlebitis and associated complications in newborn calves. Finally, as an intrauterine treatment in cases of endometritis, it showed superior efficacy

compared to cephapirine, the only intrauterine antibiotic preparation so far FDA-authorized. Due to its novel physicochemical properties, enro-C has enormous potential to treat various diseases. For example, the pharmacological/pharmacokinetic differences of enro-C with respect to reference enrofloxacin, can be revised elsewhere (2). Among such differences, it stands out that enro-C reaches exceptionally high maximum serum concentrations (Cmax) after intramuscular administration, giving place to an outstanding ratio of Cmax/MIC (2). With such data and applying Monte-Carlo simulations, it is revealed that enro-C could have higher efficacy against many bacterial diseases in challenging scenarios. For example, treating clinical cases of leptospirosis (3). Cows affected by this disease and treated with enro-C (15 mg/kg/day for five days), became PCR leptospira-negative on day 5, and microagglutination tests titers dropped on day 28, and gestation rates were recovered.

Clearly, the veterinary pharmaceutical industry faces particular risk in investing resources to carry out research and development of new antibiotics. Developing new antibacterial drugs, traditionally entails sizeable financial investment linked to a considerable loss of potential active principles. It is then understandable that the productivity of pharmaceutical research and the development of new antibacterial drugs has steadily declined over the last decades. Additionally, it has been said that for a novel drug to develop from bench-scale to industrial one and be available on farms, at least ten years or more is required. In Latin American countries, this can be even longer, and it may also be a more complicated process owing to a lack of installed capacities and an incipient tradition in generating novel active ingredients. The results obtained with enro-C can be taken as a new and pragmatic approach to generating new active principles at a much more reasonable cost and in a much shorter time. Enro-C is now a joint venture between UNAM and Laboratorios Aranda S.A. de CV, and commercial products with enro-C as an active principle are already available for Veterinary Clinicians in Mexico.

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Genetics

Using genomics to reduce methane emissions in livestock

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The global relevance and concern about climate change make it necessary to implement genetic improvement programs in order to reduce the carbon footprint of livestock and improve their adaptation to changes in temperatures around the world, which are expected to continue increasing. Therefore, livestock farming faces a significant challenge in the coming decades to meet the objective of reducing methane emissions by 30%, as agreed at COP26. (The 2021 United Nations Climate Change Conference). Breeding based on genetic improvement projects can contribute to reducing methane emissions from livestock. Historically, the objectives of dairy cattle breeding have focused on maximizing profitability, to having animals with high productive levels, and in recent decades it has sought to include a balance with characteristics of fertility, longevity, and resilience, seeking optimal animal welfare. However, due to society's growing concern about sustainability and environmental impact, discussion forums have been created on how to include sustainability, pollutant reduction and adaptation in livestock breeding objectives. For this, it is necessary to evaluate current genetic improvement programs and their impact on sustainability and greenhouse gas production. New traits must be included in the improvement objectives and new selection methodologies must be proposed to achieve faster genetic gain. Some of these traits are not routinely recorded on farms, and great efforts must be made to phenotype these traits, such as methane emissions or nitrogen accumulation. However, selective breeding faces unclear policies that pose uncertainty in the design of long-term breeding goals. This uncertainty prevents more direct strategies to include direct mitigation strategies in the genetic improvement objective. The recent global methane commitment aims to limit methane emissions by 30% in 10 years compared to 2020 levels. The strategies that have been proposed are: reducing the number of livestock, reducing the consumption of meat and dairy, use of feed additives, manure management and biogas production, change in methane emission standards for oil and gas producers.

Methane (CH_4) is a gas produced mainly by bacteria that live in soil, water and the stomachs of ruminant animals (mammals that have a stomach with four compartments that ferment food as an important part of the digestion process). Methane is considered a greenhouse gas (GHG) because it can trap infrared radiation in the atmosphere, causing an increase in air temperature. Methane is the second most abundant human-produced GHG in the world, after carbon dioxide (CO_2), which largely comes from the combustion of fossil fuels. Methane, once emitted, will exist in the atmosphere for 12 years, which is shorter than the lifespan of CO_2. However, methane can trap more radiation compared to CO_2, resulting in a 28 to 34 times greater global warming potential. Overall, methane represents 16% of the annual GHGs emitted into the Earth's atmosphere based on its global warming potential. Methane can be emitted from both natural and artificial sources. Wetlands represent 30% of natural sources. The high-humidity, low-oxygen conditions of wetlands are ideal for methanogens (methane-producing bacteria) to break down dead plant material. However not all types of land are methane emitters. Dry highland soils act as drains (the soil consumes more methane than it emits) for atmospheric methane that soil bacteria use as a carbon source. Overall, man-made methane emissions are greater than those from natural sources and account for 58% of the total methane

entering the atmosphere. Man-made methane contributions include emissions from enteric fermentation, natural gas and oil production, landfills and solid waste, rice cultivation, wastewater, manure management, biomass combustion, coal mining, static and mobile combustion, and other agricultural activities.

Globally, agriculture (enteric fermentation, manure management, rice cultivation and other agricultural activities) contributes 29% of human-generated methane emissions. Livestock is the largest single source, accounting for 73% of agricultural methane emissions (Knapp et al., 2014).

Reducing enteric CH4 in ruminants without altering animal production is desirable to reduce the carbon footprint of livestock and to make production more profitable by having more efficient animals. The genes associated with production and growth can be explored and targeted selection can be made (Manzanilla-Pech et al., 2022), to have more productive animals with less environmental impact. Basically, total CH4 emissions will decrease if annual milk production remains constant and fewer cows are needed to produce the same amount of milk (Lassen and Difford, 2020). In addition, management practices that increase yield and reduce methane must be improved, such as the addition of additives that maximize bacterial fermentation, improve diet quality, and increase the amount of fiber.

The first point to start a genetic improvement program is phenotyping, so it is very important to establish how the characteristic will be measured routinely, and that phenotype must be repeatable and have a heritable factor so that there can be a response. to genetic selection. The ways to register CH4 are:

1. Respiratory chambers (RC), which are the reference method: They are very accurate and can be used in small-scale experiments, however, they are difficult to use and do not represent natural environmental conditions. Assuming a single day of acclimatization and two consecutive days of recording, a single camera can record the CH4 production of 120 cows for a year. In practice, this number is likely to be much smaller (30 to 50 cows) per year, as reported in a large-scale genetic evaluation of CH4 emissions from 1,042 growing Angus steers (Donoghue et al., 2016a). . This study showed that CH4 production is repeatable (t = 0.97) over consecutive days, heritable (h2 = 0.27 ± 0.07) (Donoghue et al., 2016a; b) and has a moderate genomic prediction accuracy of 0. 32 ± 0.04 (Hayes et al., 2016).
2. “Sniffers”; NDIR (Guardian NG Edinburg Instruments Ltd., Livinstong, UK), measures CH4 concentrations (ppm) in breath or exhaled air. They can be installed in commercial farms, thousands of animals can be measured at the same time. Time and investment is lower, they do not affect the behavior of the animals, however, their precision and accuracy are lower than the standard method. The Sniffer samples methane emissions when cows are milked; Consequently, the data are not necessarily representative of methane emissions for a full day. Additionally, the lack of flow information (active airflow with measured volume) means that methane concentration is recorded, not production. Researchers then use calibration equations (using weight and milk production data) or recovery factors to estimate CH4 production.
3. Sulfur hexafluoride (SF6) technique, GreenFeedTM: Known as SF6, because it uses this gas as a marker, a capsule is introduced into the rumen that releases SF6 at a constant and known rate, which is expelled from it. The way methane and in mixture with it, for this a permeable tube (capsule) is used that contains SF6 gas, which is placed inside the rumen of the animal days before starting the measurement phase. A device is placed on the animal to capture and store the collected gas sample. Afterward, the concentration of CH4 and SF6 in the samples is determined by gas chromatography. (Montenegro Ballestero and Barrantes Guevara, 2016).

Due to the difficulty of using CR in genetic improvement programs, we have sought to study the genetic correlation between sniffers and CR measurements, which is 0.77 ± 0.18 between CH4 production and CH4 production by CR, while the genetic correlation between CH4 concentration by sniffer and CH4 production by CR is 0.75 ± 0.20. To resolve the lack of precision of the sniffers, the use of repeated measurements has been proposed.

The use of genomic selection using reference populations seems to be a good alternative, however, relatively large populations with genetic relationships are needed for the evaluations to have adequate precision.

Types of phenotypes: 1) methane production as mass flow rate per day (liters or grams per day), 2) methane yield (MY), which is CH4 production divided by feed intake (e.g. , CH4 production/kilogram DMI, 3) methane intensity (MI) per unit product (e.g., CH4 production per kilogram of ECM and 4) residual methane production (RMP) (e.g., methane returned in DMI, BW and ECM). But other measures are also known for the production of methane per unit of digestible DM.

The heritabilities, by different methods of methane production, are in a range between 0.05 ± 0.07 (laser detection method), up to 0.26 ± 0.11 (sniffer), and are normally measured in Holstein populations. Regarding genetic correlations, genetic correlations between methane emissions and other characteristics have been estimated at 0.49 ± 0.12 with milk production, 0.60 ± 0.13 with feed consumption, -0.28 ± 0.10 with BCS, and -0.32 with animal health, the udder (Lassen and Difford, 2020).

Conclusions: It is extremely important to have reference populations with CH4 measurements to be able to establish genetic improvement programs through traditional or genomic selection in dairy cattle. It is important to be able to compare the genetic correlations between the methods of recording emissions of CH4. methane and the phenotypes to be used as indicators of indirect selection. Such an exercise would be invaluable in unraveling the genetic relationships between methane and existing selection traits, as well as potential new phenotypes, such as feed efficiency. Method development should continue to improve existing methods (such as sniffers), increase the scope of applications, and decrease the costs of large-scale recording (such as CR and sulfur hexafluoride (SF6) technique, GreenFeedTM). Initial findings on rumen microbial ecosystems and feed efficiency offer exciting new fields of genetic research, but require considerably larger studies in the future. Genetic selection is a powerful tool to change the level of an economically important trait. This is also the case for methane emissions, but we are not there yet. Genetic selection cannot be a stand-alone mitigation strategy and solve all problems. Other initiatives will also affect the release of greenhouse gases from agriculture. In the future, it will be even more important to collaborate across disciplines within animal science and related areas to improve mitigation strategies.

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Herd health and production medicine.

Bovine respiratory disease: update on diagnosis, surveillance and treatment

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This talk will focus on select recent (last 3 years) evidence-based information from research on metritis. Results from a recent scoping review evaluating common factors used to define clinical diagnosis and treatment of puerperal metritis will be discussed and serve as a review of core concepts. Findings from deep sequencing of the microbiome of the bovine uterus microbiome using deep shotgun metagenomic analyses will be presented, which compared cows without metritis, cows with metritis, and cows with purulent vaginal discharge. Furthermore, whole genome sequencing data challenging concepts on *Escherichia coli* role in metritis pathogenesis will be discussed. We will also dive into recent findings on the diagnosis and treatment of metritis, including ground-breaking concepts on the selective treatment of metritis based on the prediction of cows that will spontaneously cure from metritis, without the need for an antibiotic treatment.

Insights into genetic disorders in cattle

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In humans, genetic disorders are considered to be individually rare; however, they account for approximately 80% of rare disorders, of which there are numerous thousands. There is no overall definition for rare disorders. However, an average prevalence threshold between 40 and 50 cases/100,000 people have been assumed. By similarity the same definition might be applied to cattle. Genetic disorders might be caused by a complete or partial change in the DNA sequence when compared with the reference sequence. They might be associated with pathogenic variants in one gene (monogenic disorders often referred to as Mendelian), in multiple genes (polygenic disorders), or in combination with environmental factors that might render the individual more or less susceptible to develop certain disorders. The causal variants can be single nucleotide variants (SNV) (e.g. missense variants), small indels or larger structural variants (e.g. chromosomal abnormalities such as the trisomy or monosomy).

Most of the reported genetic disorders in cattle follow a monogenic pattern. Several basic modes of inheritance exist for these disorders including autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive. However, not all genetic conditions follow these patterns, and other rare forms of inheritance such as mitochondrial inheritance may exist.

The first reports of recessive lethal disorders in cattle were published in 1928, several decades before the structure of DNA had been described. In the last decades, the molecular genetics has evolved in a tremendous manner especially with the inventions of second- and third-generation sequencing technologies allowing a rapid and cost-effective whole-genome sequencing (WGS). Unlocking the secrets of the cattle genome in 2009 provided the creation of the first draft of a bovine genomic assembly based on DNA from the inbred Hereford cow Dominette. The availability of the bovine genome sequence represented an important evolutionary step for learning and better understanding genetic disorders in cattle. Currently, more than 260 Mendelian diseases have been reported in cattle. It can be said, that thanks to the development and continuous improvement of the cattle assembly, the constant advance of molecular genetics methods, as well as the detailed phenotypical descriptions and active participation of stakeholders, all types of genotype-phenotype associations can be potentially investigated. In fact, the significant advances in their knowledge that have been done in the last decades are allowing the adoption of structured programs for their control.

Traditionally, inherited diseases in cattle have been investigated opportunistically, based on their sporadic occurrence and subsequent targeted analysis of affected individuals. In this respect, the authors apply a systematic protocol for phenotype characterization, which includes:

- case and herd history and anamnesis (including pedigree);
- clinical examination;
- ancillary diagnostics (e.g. blood analysis, ultrasound, radiographs, etc.);
- exclusion of environmental factors that could produce phenocopies (e.g. BVD, SBV and BTV intrauterine infections associated with congenital malformations);
- gross pathology (where appropriate);

- histopathology (where appropriate; obtained from biopsy or necropsy).

This approach is known as the forward genetic approach (FGA) and some examples of diseases identified with this approach will be given during the presentation.

However, the above mentioned sequence of investigations is only possible if suspected cases are referred to diagnostic centers.

Modern genomic technologies, such as single nucleotide polymorphism (SNP) array genotyping and whole-genome sequencing (WGS), allow straightforward locus mapping and identification of candidate causal variants in affected individuals or families. Therefore, the reverse genetic approach (RGA) is used to screen for underlying potential lethal or sub-lethal variants. This approach requires the availability of massive population-wide genomic data. By applying a haplotype screening for significant deviation from Hardy-Weinberg equilibrium, genomic regions potentially harboring candidate causal variants are identified. The subsequent generation of WGS data from haplotype carriers allows for the mining of pathogenic variants potentially causing a reduction in homozygosity. The identified putative deleterious variants for some phenotypes can be validated by phenotypic characterization of the identified affected animals.

Regardless the approach, the identification of candidate causal variants can be used as a tool for precision diagnostics and represents a step towards precision medicine in cattle. In addition, recessive variants can be easily genotyped and allow targeted breeding to reduce the number of at-risk mating, and consequently a reduction in the number of affected animals and a significant improvement in animal health and welfare.

Control of digital dermatitis in cattle: an evidence-based approach

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Lameness is the 2nd or 3rd most common disease in cattle in order of frequency and from an economic point of view (after metabolic disorders and mastitis for dairy cattle). The frequency of lameness is also increasing in beef cattle. The economic losses due to lameness are considerable: early culling (including bulls in natural service), reduced milk production and growth performance, and reduced reproductive performance. A lame cow and a DD-affected cow cost €307.50 ± 8.40 (SD) and €391.80 ± 10.0 per year on average, respectively. The results also showed a cost of €12.10 ± 0.36 per week-cow lameness. Lameness is also a major threat to animal welfare. While early intervention on a lame animal usually only requires limited and inexpensive care, a few days' delay significantly increases costs.

The issue of controlling Digital Dermatitis (DD) remains topical, with between 75% and 100% of dairy herds being affected in Europe and North America, and the disease being increasingly reported in beef herds. However, within-herd prevalence varies widely (from 1% to 75%), demonstrating that although eradication of the disease remains exceptional, it is possible to control it to an acceptable level. However, from the outset, it is important to note that there is no miracle solution. An understanding of the origin and transmission of the disease, the effects and limitations of anti-infective treatments, and the husbandry practices that can influence DD are all necessary to be able to advise on a control strategy suited to each situation.

This keynote aims to outline the main recent advances that can help us to control DD more effectively, to propose a case management approach for cattle and, ultimately, to present a global control strategy, under the acronym FIGHTERS, initially proposed by Arturo Gomez et al.

What acronym for what meaning

This is the FIGHTERS strategy.

- Footbath: Foot bath

- Infection Status: Frequency and type of lesions

- Group of animals

- Hygiene

- Trimming

- Early topical treatment: Early detection and treatment

- Record Keeping

- Skin Quality: Strengthening skin health and protection

This acronym fits perfectly for DD, but is not fully adapted for non-infectious causes of lameness. An adapted FIGHTERS can then be proposed (Table 1.)

Table 1. Presentation of the FIGHTERS strategy for both infectious and non-infectious case of lameness

Infectious Lameness	Not infectious lameness
Footbath	Feeding
Infectious status/immunity	Identification claw lesions
Groups of animals/Genetic	Groups of animals/Genetic
Hygiene	Housing
Trimming	Trimming/Traumatism
Early detection and treatment	Early detection and treatment
Record keeping	Record keeping
Skin Quality	Standing time / Slipping floor

More in details the different aspects of the FIGHTERS strategy are displayed in Figure 1.

Figure 1. The Fighters Strategy (adapted from Gomez et al.)

Without going into detail on each of these points (which will be the subject of forthcoming articles), we can nevertheless already formulate some key points for each sector.

Footbath: take care about dimensions, renewal and compliance

- Design: so that cattle can dip their entire foot in the footbath several times, the footbath should be 50 to 70 cm wide (no need for more) and at least 3 to 3.5 m long: in practice, footbaths are often too short! (Figure 2)
- Frequency: renewal of the product (at least every 100 to 120 passes, because after that the organic matter will deactivate the product and, above all, the residual volume will not cover the entire foot), hence the desirable height of the foot bath of 25 cm. Then, whatever the product, it is high and above all regular frequency that will ensure effectiveness (often 4 consecutive milkings every 15 days).
- Products: to date, we have very little data to justify or prefer one product over another. Regular foot bathing on clean feet seems to be the key

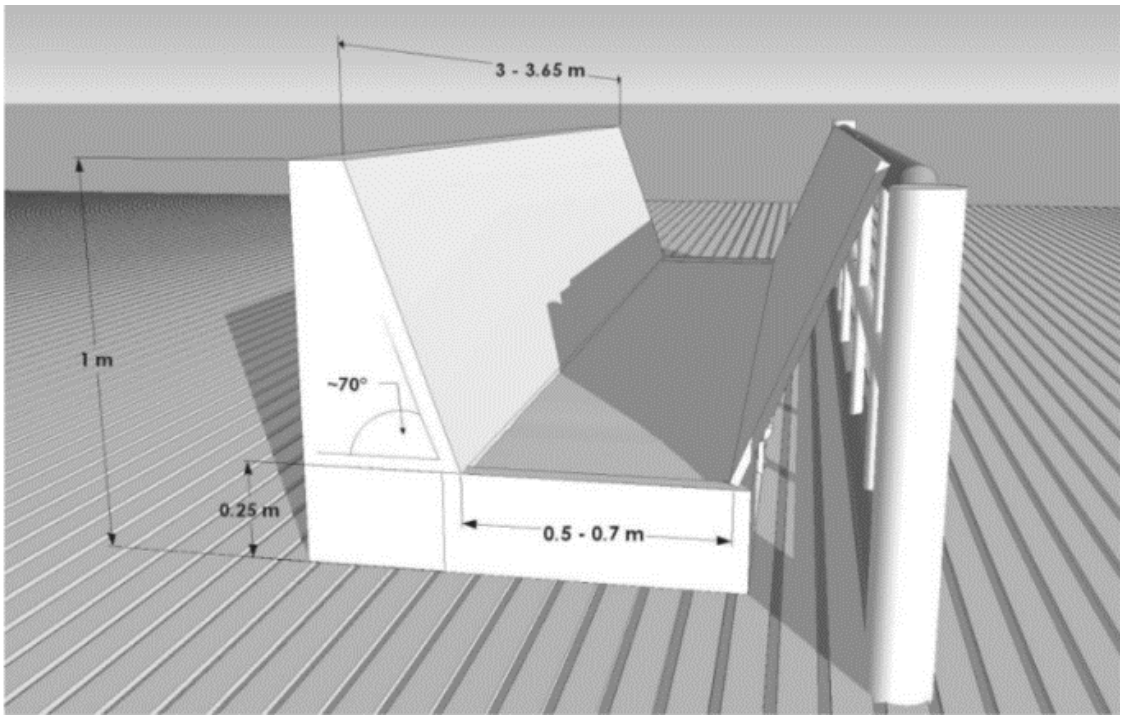

Figure 2. Ideal" foot bath dimensions to maximise the number of foot soaks while using the least amount of product (according to Arturo Gomez and Cook).

Infectious status: regular observation of lesions in the herd

- Prevalence: Lifting the feet regularly in a trimming chute would be ideal; the alternative is observation with a mirror in the milking parlour. Identified lesions should be treated.
- Evolution: it is important to see how the lesions evolve: very red and inflammatory lesions (acute) should be treated, more chronic lesions such as M4 (see photo) no longer cause the animal to limp but are a major reservoir of germs that cause DD. These animals should be targeted for culling if there are too many. The use of the M5-stage scoring system should be promoted (figure 3).

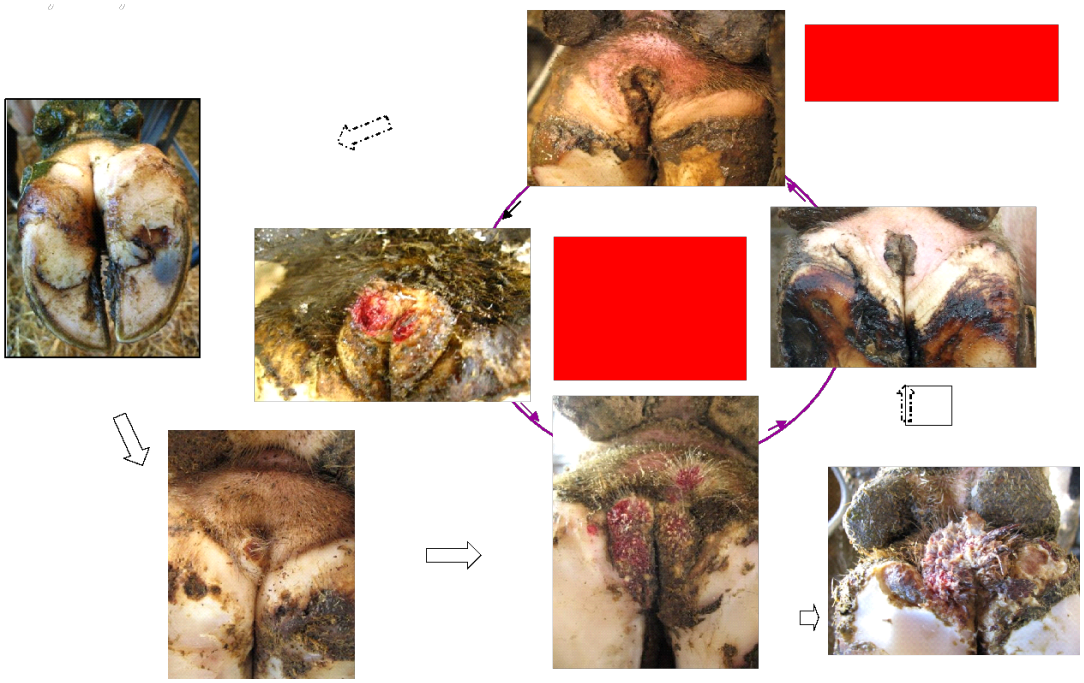

Figure 3. Illustration of the M5-DD scoring system (adapted from Döpfer)

Groups of animals: don't forget to look at heifers

- Cows: cows need all your attention, as they have the most dramatic impact (lameness scoring, lesion scoring). Regular inspection is the key. Considering Genetic resistance against DD is also promising.
- Heifers: but don't forget the heifers. In affected herds, heifers are lesions and studies have shown that heifers arriving in the dairy herd with lesions (i) risk recontaminating the cows and (ii) will give less milk and have lower fertility than heifers arriving without lesions/ The pre-herd must receive the same attention as the cows.

Hygiene: the key to control, Hygiene, Hygiene and more Hygiene

The infectious origin of DD was recognised fairly early on, due to the contagious nature of the disease and the clinical healing of lesions following the administration of antibiotics. Numerous studies conducted since then have shown that DD is polymicrobial in origin, with 3 treponemal phylotypes playing a key role: T. phagedenis-like, T. pedis (formerly T. putidum/denticola-like) and T. medium (T. vincentii)-like. These treponemes have been systematically isolated from DD lesions, alone or in association, even in the deep layers of the epidermis, and do not form part of the cutaneous flora of healthy feet. They also predominate in the microbiota of active DD lesions.

However, these treponemes are not capable of inducing DD lesions on their own. Initial trials only succeeded in experimentally reproducing DD lesions by applying a DD lesion grind to skin that had been scarified and macerated beforehand. A break in skin integrity is therefore necessary. In animal husbandry, these conditions are met when the feet are macerated in faecal matter from muddy areas, or when pebbles or abrasive structures injure the digital skin. Recently, a new study showed that DD could be reproduced with a lesion grind but not with

pure cultures of treponemes associated with DD, after creating micro-scarifications and macerating the digital skin. This suggests that several microorganisms are needed to facilitate the penetration of DD-associated treponemes or contribute to their pathogenicity, with skin maceration being the key factor.

- The first thing to do is score hygiene: check that feet are clean at the feed bunk or in the milking parlour. The bacteria that cause DD on a dry, clean foot will not lead to developing lesions. A moist, macerating foot is more likely to cause lesions. Cleanliness is the key. Regularly washing the feet is in itself a good prevention method.
- Then, if the feet are dirty, it's a good idea to look for the critical points, the number 1 enemy of cleanliness being dampness: location/leakage of water troughs, ventilation, frequency of scraping, slopes, etc. Areas near water troughs are often an area for improvement.

Trimming: trimming is not just for hoof diseases

- Preventive trimming: animals suffering from DD will change their balance and horn growth will be affected. Correct footing also means putting some distance between the heel (the preferred area for DD) and the ground. Well-maintained feet are more resistant to DD.
- Curative trimming is also important: animals suffering from DD will have a better chance of recovery if their feet are trimmed and a dry bandage is applied and changed regularly.

Early topical treatment: treat as soon as possible, as long as needed

- Firstly, detect the animals affected: by regularly spotting lame animals or lesions in the milking parlour, at least once a week and twice a month.
- Then apply a topical treatment (no systemic antibiotics!!) based on a disinfectant or antibiotics with marketing authorisation as your vet prescribes.

Record keeping: data are crucial for monitoring DD and efficacy of control actions

- To know whether what you're putting in place is working, you need to be able to count things (lesions, treatments, etc.). So recording this information correctly, ideally using your business software, is essential.
- Armed with this data and its monitoring throughout the year, you can identify periods of greater risk, which may lead you to change your practices (increase the frequency of foot baths, for example, or bring in a horse trimmer, etc.).

Skin Quality: the animal's first line of defence

- If the skin is healthy, free of lesions and dry, there is little chance of DD lesions. So it's important to provide animals with a good supply of vitamins, minerals and trace elements (such as vitamin A and zinc) that play a role in skin quality.
- But the best way to protect the skin is still to keep your feet dry: so hygiene, hygiene and more hygiene!

In conclusion, when it comes to controlling DD, there is no single flagship measure or miracle product, but rather a multi-factorial approach to this scourge of our livestock farms (Figure 4).

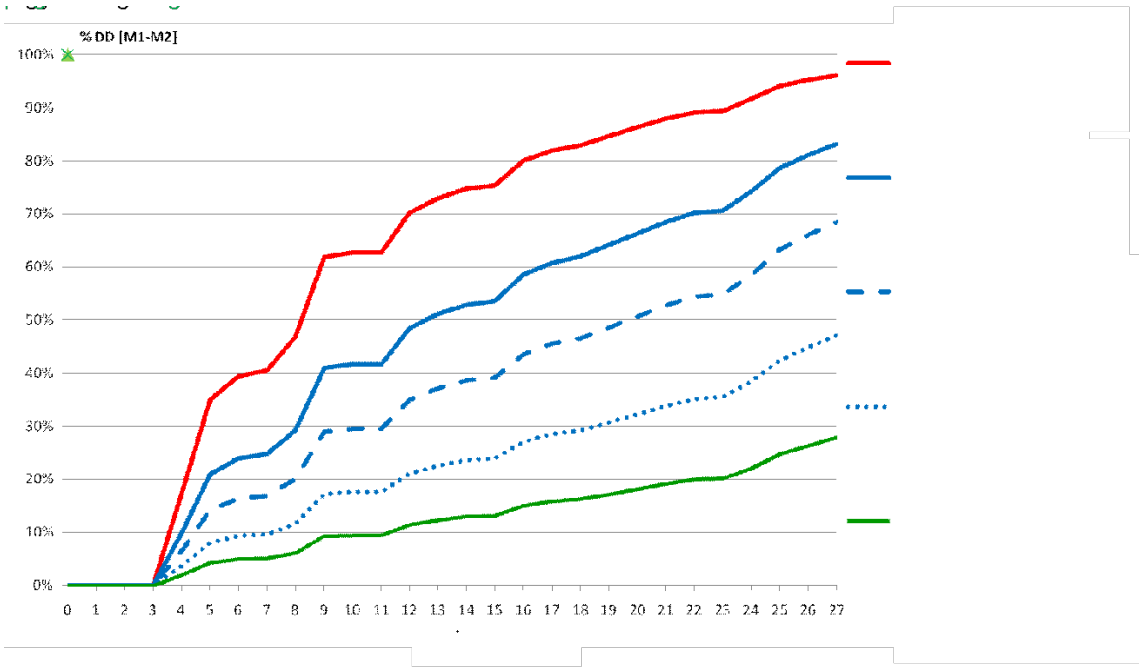

Figure 4. Modelling of the efficacy of different control actions alone or in combination) to prevent DD lesions.

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Production impacts and resistance of gastrointestinal nematodes in feedlot cattle

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Control of gastrointestinal parasites is essential to the health and productivity of cattle entering feedlots. Gastrointestinal parasites have been shown to negatively impact average daily gain of feedlot cattle. *Cooperia punctata* significantly reduced feed intake of calves fed a commercial feedlot grower ration. Hence, deworming using an anthelmintic drug is a common management procedure conducted at feedlot entry to control internal parasites.

The three classes of anthelmintics most commonly registered and used in cattle are the avermectin/milbemycins (abamectin, doramectin, ivermectin, moxidectin), benzimidazoles (albendazole, fenbendazole), and imidazothiazoles (levamisole). Globally, the most common class of anthelmintic used to treat feedlot cattle at induction are the avermectin/milbemycins due to their broad-spectrum endectocidal activity.

Control programs in feedlots are primarily based on treatment with anthelmintic drugs at the time of induction and housing cattle in an environment where transmission of gastrointestinal nematodes is minimized. Globally, there are increasing numbers of reports of anthelmintic resistance in gastrointestinal nematodes of cattle. While resistance to the avermectin/milbemycins has been reported in *Cooperia* spp., *Ostertagia,* and *Haemonchus* in cattle grazing pastures in North America, South America, Europe, and Australasia, reports of resistance from feedlot cattle are rare. Since cattle entering feedlots are sourced from grazing properties including high-intensity backgrounding operations, it is highly likely that resistance profiles reflect the parasite populations on these paddocks where feedlot cattle are sourced.

Feedlot producers are not primarily concerned regarding anthelmintic resistance, but more concerned about the potential impact of anthelmintic resistance on the health and performance of feedlot cattle. Properly randomized controlled trials evaluating the impact of parasite control programs on the growth and productivity of feedlot cattle are extremely limited. Hence, the data presented in this keynote presentation will examine the level of anthelmintic resistance in common gastrointestinal parasites entering a commercial feedlot in Australia and more importantly determine the impact of drug resistance on animal performance, health, and carcass quality and yield.

A single-blinded randomized complete block design tested the effects of six parasite control protocols including 1) no anthelmintic, 2) injectable doramectin, 3) oral albendazole, 4) oral levamisole, 5) triple combination of injectable doramectin, oral albendazole, and oral levamisole, and 6) oral triclabendazole plus triple combination of injectable doramectin, oral albendazole, and oral levamisole.

Cattle (n=1434) entered the feedlot at 404.1 ± 36.0 kg (mean ± standard deviation), gained 2.10 ± 0.39 kg/head/day for 103.7 ± 0.9 days, exited the feedlot at 621.9 ± 56.9 kg, and yielded carcasses weighing 343.6 ± 32.3 kg. Faecal egg count reduction tests

including larval cultures were completed using individual faecal egg counts. Important insights regarding methodologies to complete faecal egg count reduction tests in feedlot cattle were gained. The mean faecal egg count at feedlot entry was 77.6 ± 180.9 eggs per gram of faeces. The most common genera of gastrointestinal nematodes were *Cooperia* and *Haemonchus*, respectively representing 73% and 15% of cultures. Resistance to injectable doramectin was detected in five of six replicates, and suspected in one of six replicates with mean faecal egg count reduction ranging from 62-96%. A low level of resistance to oral albendazole was suspected in three of six replicates, while oral albendazole was highly effective in the other three replicates. Oral levamisole and both combination protocols were highly effective. There was no difference in production parameters between any of the five anthelmintic treatment protocols including injectable doramectin. However, cattle that were treated with any of the five deworming protocols had 0.06 kg/day higher average daily gain, 6.2 kg higher live exit weight, and 3.3 kg higher hot carcass weight than cattle that did not receive an anthelmintic, demonstrating the significant productivity and carcass benefits of deworming cattle at feedlot entry.

Navigating Bovine Health Status in Mexico: A Multi-Disease Diagnostic Strategy

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Introduction

Significance of beef production in Mexico for local and global markets highlights the importance of disease surveillance to safeguard animal health, as well as to ensure economic and productive efficiency. While studies conducted in Mexico consistently identify the presence of viral infectious agents such as BVD, IBR, BLV, PI3, and BRSV in cattle herds, there is a lack of official situational diagnostics to inform the epidemiological context in bovine populations. In line with current international trends, towards controlling and eliminating endemic diseases, diagnostic studies to ascertain the health status of cattle in Mexico are vital to improve epidemiological surveillance and allow for informed decision-making,

Hypothesis

The apparent prevalence of BVDv, IBRv, BLV, PI3v, and BRSV will fall within meso- (10-50%) to holo-endemic (over 75%) ranges in cattle herds across Mexico.

Objectives

Our aim was to develop and validate a highly efficient immunoassay for simultaneous detection of BVD, PI3, BRSV, IBR, and BLV antibodies, and to establish the seroprevalence of these diseases within the national herd. Additionally, we assessed environmental and production factors that could impact seropositivity and conducted geospatial interpolation analysis to identify high seropositivity zones.

Materials and Methods

We developed and validated an immunoassay using the Luminex® platform, to assess the seroprevalence of five key bovine diseases. Our study encompassed 2,686 blood samples from healthy, non-vaccinated cattle over two years old from diverse cattle-rearing regions in Mexico. Seroprevalences and relative risks (RR) for environmental and production factors were calculated. The simple Kriging method was utilized for geospatial interpolation and evaluation of seropositivity across Mexico.

Results and Discussion

An assay that can be efficiently used for the simultaneous detection of antibodies against BVDv, PI3v, BRSV, IBRv, and BLV was designed and validated. The prevalence for the studied diseases ranged from meso-endemic to hyper-endemic levels in unvaccinated adult cows, suggesting natural pathogen exposure and reflecting sanitary status in the national herd.

Our study found that cattle in humid climates seem more susceptible to these diseases, especially in the coastal areas of Mexico. Moreover, year-round breeding system, a common practice used by 97% of Mexican herds, appears to be a factor in disease

prevalence. These findings highlight the need to evaluate and modify management strategies to reduce the potential risks of disease spreading.

Geospatial interpolation identified the highest seropositivity for most diseases in northern Oaxaca and southern Veracruz. Our mapping efforts could help outline strategies initiating control programs in regions with low seropositivity and establishing sanitary barriers to prevent disease spread from high seropositivity areas.

Conclusions

The seroprevalences identified in this study emphasize the urgency for the surveillance and development of control and eradication programs. Developing effective vaccines and new diagnostic techniques would help mitigate the economic impact of these diseases and sustain Mexico's competitiveness in the global market.

Phenotyping for Dairy Cattle Resilience: Using Genetics and Genomics to Improve Livestock Sustainability

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Introduction

The dairy industry is facing several challenges related to human and animal health, sustainability, and social acceptability; simultaneously, the demand for dairy products is increasing globally (Brito et al., 2021). Efficient breeding programs can play a pivotal role in shaping more sustainable food systems in the future, in particular by balancing breeding goals for production traits with those related to longevity, fertility, health and welfare, workability, milk quality, environmental efficiency, and overall resilience (Miglior et al., 2017; Seymour et al., 2019). The Resilient Dairy Genome Project (RDGP; http://www.resilientdairy.ca) samples phenotypes related to dairy cattle resilience on a large scale. Here, resilience is defined as an animal's ability to adjust quickly to changing environmental conditions without compromising its productivity, health, or fertility, while becoming more resource-efficient and lowering its environmental load. Traits associated with closer-to-biology fertility, enhanced disease resistance, and environmental efficiency are key components of this definition. The overall objective of the RDGP is to enhance dairy cattle resilience, by phenotyping novel traits, generating genomic tools, and developing statistical methods to breed dairy cows with the capacity to adapt rapidly to changing environmental conditions.

Material and Methods

From 2015 until June 2025 data is being collected from dairy herds globally in a collaboration among research and industry partners in Australia, Canada, Denmark, Germany, Spain, Switzerland and United States. Information from 18,648 dairy cows on production, feed intake, body weight and enteric methane emissions, and milk mid-infrared spectral was combined into a database containing all records from each country (Table 1). The pedigree included 72,682 individuals, with a total of 11,819 genotyped cows. A detailed description of the database can be found in van Staaveren et al. (2024), which explains the imputation of the genotypes, their quality control and the principal component analysis, and consistency of gametic phase performed to evaluate the level of relatedness between the animals in different countries (van Staaveren et al. 2024).

Table 1. Number of records and cows for daily milk, weekly and monthly fat and protein yield, daily and weekly dry matter intake (DMI), weekly and monthly body weight, methane (CH4) emission and milk mid infrared spectra (MIR) in the RDGP database as of October 2023.

Trait	Number of records	Number of cows
Milk yield	1,227,427	18,648
Fat yield	528,313	18,366
Protein yield	528,453	18,367
DMI	1,508,751	14,868
Body Weight	927,076	16,646
CH4	33,723	4,504
MIR	142,993	3,383

Results

In the ongoing RDGP, multiple studies have been published, including investigations on traits associated with closer-to-biology fertility, enhanced disease resistance, and environmental efficiency, which are key to provide research solutions to breed a more resilient dairy cow. In the following sections, we give a comprehensive overview of the published studies performed to genetically enhance dairy cattle resilience as part of the RDGP.

Closer-to-biology fertility. Fertility traits are highly influenced by environmental effects. The success of pregnancy, for example is influenced by the intensity of estrous expression, the embryos stage of age (Madureira et al., 2021a) and the progesterone levels at conception rate (Madureira et al., 2021b). Therefore, it is crucial to identify high-quality traits for genetic evaluation and their quantitative trait loci (QTL) for understanding better their genetic architecture and genetic relationships. To investigate novel fertility traits that are closer-to-biology, genetic parameters of the reproductive tract size and position score

(SPS) were estimated as a possible novel fertility trait (Martin et al., 2022a). As an indicator for pregnancy rate, number of services per pregnancy, and pregnancy loss, SPS is based on transrectal palpation of the reproductive tract. Preliminary results showed SPS to be heritable, with no relationship between SPS and production traits and favorable relationships to fertility traits (Martin et al., 2022a). Anogenital distance is defined as the measurement of the distance from the center of the anus to the genital and a promising indicator in females for favorable reproductive outcomes, including an increase in pregnancy per artificial insemination and a decrease in age at first insemination. Genetic parameters of anogenital distance show the possibility to select on this trait (Dodd et al., 2024). However, gametic incompatibility might reduce the odds of fertilization and the probability of pregnancy. Based on recognition (i.e., sperm-egg recognition, cell-

cell recognition) and immunological functions within the reproductive tract, Martin et al. (2022b) showed that fertilization is not always a random event as commonly assumed. The industry should consider gametic incompatibility in the future, because the animal's probability of becoming fertilized is not only predicted by its innate fertility, but also by its mate gametic compatibility. Therefore, this study provided information that could be used to increase the possibility to predict the mating success (Martin et al. 2022b). In addition to studying32 optimal mating decisions, Alcantara et al. (2022) produced robust models that correctly identified timed artificial insemination protocols from dairy cattle records using machine learning algorithms. Thus, opening the possibility for unbiased genetic evaluation of animals based on their natural fertility (Alcantara et al. 2022). A genome wide association study (GWAS) was performed to identify QTL and candidate genes associated with fertility and reproduction traits in North American Holsteins (Chen et al., 2022). Hereby, QTL were broadly distributed across the entire genome for heifers as well as for cows, which are consistent with the polygenic nature of fertility and reproduction traits and multiple novel candidate genes were detected. The study reported a comprehensive investigation of the underlying genetic basis of fertility and reproduction in heifers, cows, and sires, improving the understanding of the genetic control of fertility and reproduction traits in Holstein cattle (Chen et al. 2022).

Enhanced disease resistance. To breed more disease resistant animals, accurate farm-level disease phenotypes are needed. In van Staaveren et al. (2023), the current state of calf disease recording for genetic evaluation in Canada was evaluated. Focussing on calf diseases, such as respiratory illnesses and diarrhea, farms collect these events more frequently than other calf diseases. However, a pipeline between farms and official organisations performing genetic evaluation is still needed. This would include standardized case definitions for the illnesses, as well as standardized operating protocols for recording and data exchange, to assist with consistent recording of calf diseases and to start including calf health traits in genetic evaluations (van Staaveren et al. 2023). Heritability estimates for disease traits are quite low, however, Lynch et al. (2023) highlighted the possibility to select for reduced diarrhea and respiratory diseases in dairy calves, as substantial genetic variation exists. Currently genetic correlations to other important traits are estimated (Lynch et al., 2024) and possible QTL and candidate genes are investigated for diarrhea and respitatory diseases for genetic evaluation (Lynch unpublished). Enhancing disease resistance further, Bongers et al. (2022) estimated the trait enzootic bovine leucosis to be heritable. To select against fertility disorders in Canadian dairy cattle a multivariate approach including the traits cystic ovaries, metritis, and retained placenta was reported by Jamrozik et al. (2021) and used for genetic evaluation.

Environmental efficiency. Currently one of the most pressing topics is to enhance modern farm systems' sustainability. Breeding programs might help reduce the environmental footprint from cows, by selecting more feed and methane-efficient animals and to select more adaptable cows to the changing climate. Dairy cattle in North America face major problems adapting to heat, where heat stress can have negative effects on their overall health, as well as their production. As a first

step to investigate the tolerance to different heating loads, studies of Campos et al. (2022a) and Rockett et al. (2023) used NASA POWER estimates of weather parameters to calculate the temperature-humidity index (THI). To estimate phenotypic heat stress thresholds in production traits, THI was used as a covariate. High temperatures had a negative effect on the production traits, i.e., milk-, fat-, and protein yields in Canadian Holsteins across all regions in Canada (Rockett et al., 2023). Moreover, the genetic merit of these traits depends whether or not the animal is evaluated under thermal comfort or heat stress (Campos et al., 2022b).

To enhance only the cattle's adaptability to the changing environment is however one-sided. There is a need to breed for feed and methane efficiency for more sustainable food systems in the future. The ever increasing feed costs pressure the industry to select more feed-efficient cows. Using simulated data, Houlahan et al. (2021) showed that selecting on feed conversion efficiency directly allowed for an economically favourable and a more balanced response to selection. If too much weight is placed on the breeding values of feed efficiency, there might be negative implications for other traits (Houlahan et al., 2021). Important traits, such as dry matter intake, energy corrected milk, metabolic body weight, and feed efficiency can be measured multiple times for each cow. Their corresponding genetic parameters change over time as well, which needs to be considered in the models used for genetic evaluation. Moreover, improving feed efficiency at optimal stages of lactation may be feasible in dairy cattle (Houlahan et al., 2023). Underlining this hypothesis, Bolormaa et al. (2023a) showed that dry matter intake and residual feed intake sampled in late lactation captured more genetic variation and increased genomic prediction accuracy. Genomic prediction of this trait could be even further improved by including genomic variants (Bolormaa et al., 2023b). Due to these research findings of the project, it was possible to integrate feed efficiency for first and second lactation into the Canadian genetic evaluation by Lactanet (Jamrozik et al., 2022). Feed efficiency is derived as residual feed intake measured between 61 and 305 days in milk (Jamrozik et al., 2022). It still needs to be determined, however, which time periods and parities reflect on the total lactation or lifetime of dry matter intake and residual feed intake the best. Phenotyping feed efficiency traits is labour intensive and very costly. Within the scope of the RDGP, Shadpour et al. (2022a) and Fizzarin et al. (2023) investigated the ability to predict dry matter intake and the related trait of changing body condition score, respectively, from mid-infrared (MIR) spectral information. Milk samples are recorded and analyzed routinely through milk performance test, which includes mid-infrared spectroscopy. The changing body condition score can be estimated accurately from MIR data, providing information on the cow's energy status without additional costs (Fizzarin et al., 2023). Although, Shadpour et al. (2022a) showed the possibility to predict weekly average dry matter intake, further studies are needed to utilize the estimated measure of feed efficiency that accounts for the level of production.

Dairy cattle have an environmental impact through methane emissions from enteric fermentation, and its reduction is vital for a long-term sustainable dairy industry. In the RDGP methane emissions are mostly measured using the GreenFeed (C-Lock Inc.), for which Smith et al. (2024) highlighted that behavior

has to be taken into account when phenotyping for methane emissions. Two training sessions are needed for the habituation in tie-stall conditions. Methane emission traits are heritable, including daily methane production, methane yield, and methane intensity (Kamalananthan et al. 2023). Through machine learning algorithms, Shad pour et al. (2022b) was able to predict methane emissions through MIR spectral data. Additionally, Lopes et al. (2024) estimated genetic parameters of rumination time of Holsteins as a potential indicator for methane and feed efficiency. Rumination time was heritable and genetically related to methane traits, but uncorrelated to feed efficiency (Lopes et al., 2024). The RDGP gave research solutions to the industry partners, Lactanet and Semex Alliance, for them to publish the first official methane efficiency breeding values for Holsteins in 2023. Making Canada the first country to use methane efficiency evaluations as an easy and cumulative way to contribute to the environmental sustainability of the dairy industry (Oliveira et al., 2024; Van Doormaal et al., 2023).

Conclusions

The RDGP is a large-scale international cooperation between research institutes and industry partners. Closer-to-biology fertility, enhanced disease resistance, and environmental efficiency phenotypes will be key for a more sustainable dairy industry. In the scope of this project, research solutions and new genomic tools for this key agricultural sector were provided to breed for more resilient dairy cows.

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Acknowledgements

This study was part of the Resilient Dairy Genome Project (RDGP). We gratefully acknowledge the funding and support from the Resilient Dairy Genome Project (http://www.resilientdairy.ca/funders-and-partners/) including Genome Canada (Canada), Genome Alberta (Canada), Ontario Ministry of Economic Development, Job Creation and Trade (Canada), Genome Quebec (Canada), Genome British Columbia (Canada), Lactanet (Canada), Dairy Farmers of Canada (Canada), Aarhus University (Denmark), The Council on Dairy Cattle Breeding (United States), Qualitas AG (Switzerland), USDA – Agricultural Research

Service (United States), Allflex Livestock Intelligence (United States), Afimilk Ltd. (Israel), Nedap NV (The Netherlands), Illumina (Canada), Ontario Genomics Institute (Canada), University of Guelph (Canada), University of Alberta (Canada), Université Laval (Canada), University of British Columbia (Canada), McGill University (Canada), Agriculture Research Department of Jobs, Precincts and Regions, La Trobe University (Australia), Instituto Nacional de Investigacion y Technologia Agraria y Alimentaria (INIA, Spain), Confederación de Asociaciones de Frisona Española (CONAFE, Spain), Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE, France), Vereinigte Informationssysteme Tierhaltung (VIT, Germany), University of Wisconsin-Madison (United States), University of Florida (United States), Purdue University (United States), University of Georgia (United States), Iowa State University (United States), Michigan State University (United States), University of Calgary (Canada), University of Prince Edward Island (Canada), Alberta Agriculture & Forestry (Canada), Agriculture and Agri-Food Canada (Canada), Freie Universität Berlin (Germany), São Paulo State University (Brazil), GenTORE Project (France), Semex (Canada), Foundation for Food and Agriculture Research (FFAR, US), Ministry of Agriculture Food & Rural Affairs (Canada), Ontario Ministry of Research and Innovation (Canada), ETH Zurich, Animal Nutrition Group (Switzerland), Agroscope Posieux (Switzerland), University of Kiel (Germany).

Nutrition

Whole colostrum for calf welfare and long-term productivity

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Ensuring the early consumption of high-quality colostrum containing adequate types and levels of antibodies and important nutritional and biologically active substances is the single most important factor influencing a successful adaptation of the newborn calf to the post-uterine environment. The significance of colostrum ingestion to the health of the newborn calf was first established in 1922 when it was shown that calves deprived of colostrum died from E. coli infection (Smith T. and Little R. B. 1922). Since then it was clearly demonstrated that disease protection in calves during the immediate neonatal period is totally dependent on the passive transfer of antibodies found in colostrum (Robison et al. 1988). Colostrum was shown to transfers a broad array of antibodies derived from the serum of the cow that act to protect the newborn until it can mount an effective secondary immune response on its own. The level of passive transfer of immunity by colostrum consumptions is directly related to the concentration of antibodies in the colostrum, the volume of colostrum consumed, and the absorptive capacity of the calf. For many years to follow it was believed that the benefits to the calve were exclusively linked to the passive transfer of antibody and the term failure of passive transfer (FPT) was coined to describe calves that had serum IgG levels below 10 g/L (NAHMS 1994). Studies also showed that in a normal calf, uncompromised by stress or other conditions, this serum level of passably transfer antibodies could be attain by feeding colostrum containing 100 g/L within the first 6 hours of birth (Quigley III and Drewry 1998).

As consequence of these early observations, colostrum management practices were mostly directed to provide sufficient amount of immunoglobulin in order to reduce prevalence and severity of infections and reduce mortality during the first 3 weeks of life. More recently, the benefits of timely colostrum ingestion in the health of the newborn calf have been established in three primary areas of impact. The first area indeed relates to the immunological protection of the neonatal calf against infectious agents during the first weeks of life. The second area relates to the contribution of colostrum as an immediate source of energy, readily available and essential for the survival of the calf during the first hours of life. And the third and least understood area of impact of colostrum ingestion in the calf is the effect of colostrum in the long-term productivity of the animal. The presentation will largely be focused in these last 2 areas.

It has become evident that the nutritional value of the first milk is as important as the well recognized role of colostrum in immune protection against infectious agents. Energy reserves such as glycogen and fat, which are available at the time of birth are very limited in the newborn calf. It is estimated that these limited reserves are depleted during the first 3 hours of life and that these energy sources may be able to fulfil the energy needs of the calf for approximately 12 hours (Girard et al. 1992). It has been

shown that approximately 20 % of the solids in good quality colostrum are highly digestible and readily metabolically utilizable milk fats. Thus, improved anabolic metabolism observed immediately after birth in the newborn calf is directly related to high amounts of colostrum components, which provide substrates for gluconeogenesis and protein synthesis (Girard 1986, Lepine et al. 1991). Furthermore, studies comparing the metabolic status of colostrum fed calves with that of milk replacer fed calves have shown that the improved metabolic status achieved by calves fed colostrums is not achieved by calves fed milk replacer (Hammon and Blum 1998, Blum et al. 1997). In a more recent study it was shown that feeding a full fat colostrum derived colostrum replacer versus a partially defatted form had a positive effect on thermogenesis, led to higher body weight and body weight gain within 127 d of age (Valldecabres et. al. 2022). It has also been shown that the body temperature of a newborn calf increases by 15 % 1 hour after colostrum consumption (Kurz and Willett 1991). Therefore, the consumption of high-quality colostrum during these early metabolic adjustments should be considered as critical in the life of a calf.

At birth, the newborn calf is forced to adapt to receiving all nutrients through oral intake. In order to achieve this, the cells of the gastrointestinal (GI) epithelium must undergo major
differentiation/maturation changes to develop digestive and absorptive capabilities. This process is controlled by genetic programming and is influenced by a number of extrinsic factors (Pácha J, 2000). Several studies have shown that the GI epithelium of colostrum feed calves was better developed than that of non-colostrum feed animals (Roffler B et al. 2003 and Blättler U et al. 2001). Better GI epithelial differentiation may explain the improved absorption of carbohydrates observed in colostrum feed calves (Roffler B et al. 2003, and Schottstedt T et al. 2005). Thus, it has been postulated that the differentiation/maturation and absorptive properties influenced by colostrum are largely due to the regulatory properties of hormones and growth factors present in colostrum.

Taking together the importance of colostrum ingestion on both the metabolic adaptation and the immune protection it should become evident that the wellbeing of the calve depends on good colostrum feeding practices. In fact, it can be argued that failure to provide sufficient amounts of whole colostrum soon after birth could potentially trigger 5 of the identified noxious welfare experiences in the newborn which include; hunger, hypothermia, respiratory distress, sickness and pain (Figure1).

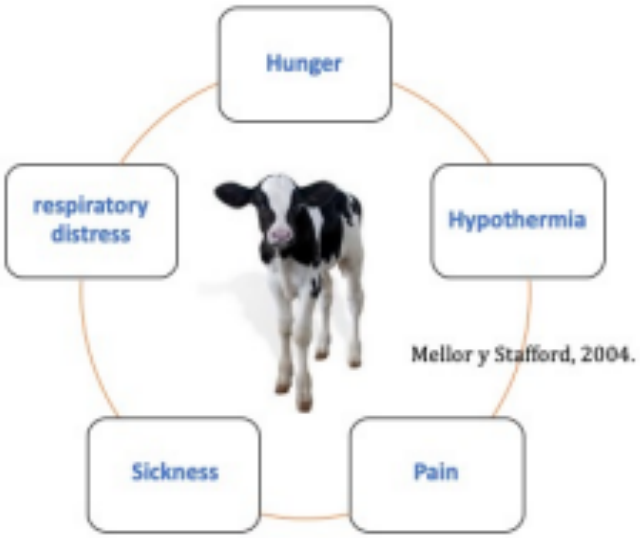

Figure 1. Poor colostrum management compromises calf welfare

It is generally accepted that serious illness in a calf at a young age is very likely to lead to life long poor productivity but there is also a growing amount of evidence that the association between colostrum intake during the first critical hours of a calf's life and long term productive performance is due to other critical non-immunoglobulin factors present in colostrum. We will discuss how colostrum feeding correspond to a critical moment in the developmental programing of the newborn and during the presentation we will use the term lactocrine signaling to describe the observations that the newborn calf is programmed by biologically active factors present in colostrum and that this signaling is a very important part of the adaptation process of the newborn to the post-uterine environment (Bartol FF et al. 2013). Although epigenetic programming can occur at any stage development we will argue that these early colostrum signals induced changes that affect developmental trajectory with lasting consequences and are in fact part of the epigenetic signals that will determine the productive phenotype of the adult animal.

We will also provide examples of how surrogate indicator of the quantity and quality of colostrum ingestion have been used in the field to demonstrate the positive effects of colostrum feeding in long term productive performance. These examples will provide support for the assertion that only whole colostrum can ensure calves achieve a state of wellbeing and then link these early events to the long-term productivity of the calf and the financial soundness of the operation (Figure 2).

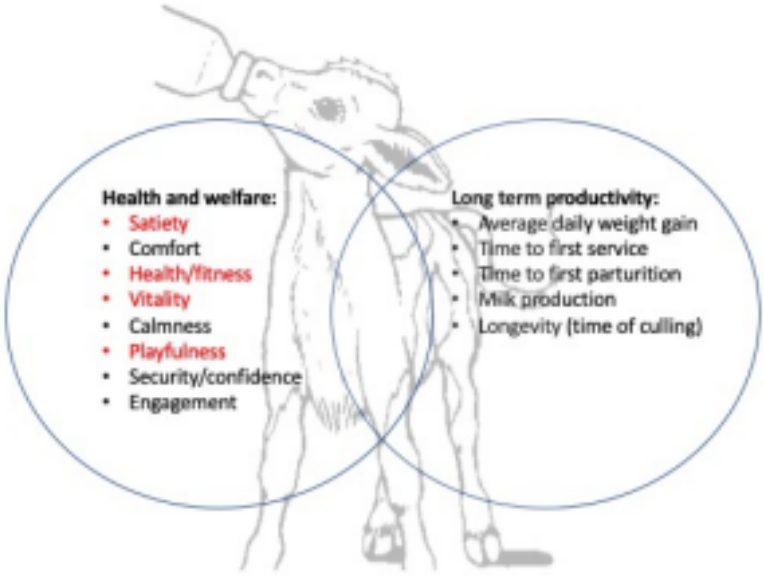

Figure 2. Relationship between calf heath and welfare and long term productivity

Higher serum levels of IgG shortly after a calf's birth (as an indicator of good colostrum feeding practices) or feeding larger amounts of colostrum have been correlated with higher milk production later in life (DeNise SK et al. 1989, Faber S. N. et al. 2005). Similarly, a significant correlation
between serum levels of IgG in calves 24-48 hours after birth and average daily gain, independently of disease status have been shown in several investigations (Robison J. D. et al. 1988, Massimini G. et al. 2006 and Dewell R.D. et al. 2006).
The fact that colostrum intake in a newborn influences milk production later in life has obvious economic implications. Lower growth rates also have important economic ramifications to the dairy industry. The growth rate of heifers from birth to sexual maturity has been shown to influence the age at first calving and milk production (Clark RD and Touchberry RW 1962, Virtala AM et al. 1996, Zanton GI, Heinrichs AJ 2005). Models that analyze the economic impact of high culling rates and delayed age of first calving have demonstrated, a significant economic impact of these parameters in the dairy industry (Tozer PR and Heinrichs AJ 2001 and Clark RD and Touchberry RW 1962). Cow longevity is linked to production parameters such as milk production and reproductive performance, and the evidence presented indicates that good colostrum feeding practices have the potential to positivity influence both. Increasing dairy cow longevity would have many benefits to the industry including consumer's perception of animal welfare and sustainability as well as making it more profitable for dairy farmers.

We believe that the information provided in this presentation gives sufficient evidence to clearly stablish the link between colostrum ingestion and the wellbeing of the newborn calf and how taking care of the health and welfare of calves benefits the long-term productivity in the herd. With this established we hope farms adopt colostrum management practices to fully capitalize on the production potential of each animal.

There are certain guidelines that if follow will improve the health and welfare of the calves that in turn would help increase the long-term productivity parameters of the dairy. The most essential colostrum feedings recommendations that we propose are:

- Feed colostrum as early after birth as possible. First feeding is recommended within the first 2 hours of birth and should be followed by a second feeding between 6 to 12 hours of birth.
- Feed only good quality colostrum with a brix reading of least 25 % brix (that would be equivalent to 50 grams per liter of IgG).
- In the first feeding the calves should receive the equivalent to 10% of their body weight) (between 3 to 4 liters) and in the second feeding they should receive at least 5% of their body weight.
- Feed only colostrum from healthy dams. Colostrum donors should be negative to tuberculosis, brucellosis, paratuberculosis and mycoplasmas.
- If colostrum is not feed immediately after milking it should be refrigerated for a short-term use and frozen for long term storage. When storing the containers should be properly labeled with date of collection, donor ID, %brix and when thawing should be done gently (max 51^{O}C).

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Importance of colostrum for small ruminants

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Newborn lambs and kids must adapt quickly from a cozy, warm, and protected environment in the mother's womb, to the external, cold, with high pathogen and environment challenges of a farm (Nowak and Poindron, 2006). The transition from inside out of the ewe/doe requires the lamb/kid to regulate its own temperature. Thus, specific management in the first day of life is essential to ensure offspring survival and health (Godden et al., 2019).

Neonatal mortality in small ruminant livestock has remained at 15% over the past 40 years, and represents a significant loss of farm income, contributes to wastage, and affects animal welfare. Scientific knowledge about the biology of neonatal adaptation after birth has been accumulating but does not appear to have had an impact on improving survival. Biologically, it is clear that achieving a good intake of colostrum, as soon as possible after birth, is crucial for neonatal survival (Dwyer et al.,2015).

The main risk factors for lamb/kid mortality are low birthweight, poor maternal nutrition during gestation, birth difficulty, litter size, colostrum intake, thermoregulation, and genetics, which can all be partly attributed to their effect on the speed with which the lamb/kid reaches the udder and sucks colostrum (Dwyer et al., 2015).

Colostrum is the first mammary gland secretion and is critical for the kid/lamb survival, since it will be essential not only for the progeny nutrition and early development but also for passive immunity transfer, mother-offspring bond, maintenance of body temperature, growth factors, antimicrobial and anti-inflammatory agents acting as bioactive components, laxative to aid meconium defecation and gastrointestinal tract microbial colonization (Hernández-Castellano et al., 2014, 2018; Akers, 2016; Arshad et al., 2021; Polidori et al., 2022).

Thus, colostrum management practices result in both short- and long-term improvements in animal health, performance, feed efficiency, and life expectancy (Banchero et al., 2004; Faber et al., 2005; Nowak and Poindron, 2006; Agenbag et al., 2021).

Small ruminants have a syndesmochorial placenta, this means that the endometrium has an intermittent exposure of maternal capillaries to the chorionic epithelium, separating fetal and maternal blood supplies, forming a placental barrier, and allowing limited passage of maternal proteins such as immunoglobulins (Senger, 2015). For this reason, newborn small ruminants are considered hypogammaglobulinemic (Hernández-Castellano et al., 2014). Therefore, colostrum ingestion will provide all the beneficial

support for nutrition, immunity, and temperature maintenance. However, acquiring passive immunity through immunoglobulin, especially IgG, is a race against time. Immunoglobulin absorption in the small intestine decreases with time, occurring most rapidly during the first six hours, and ceasing around 24-36 hours after birth (Weaver et al., 2000; Moore et al., 2005). Thus, providing good nutritional and immunological quality colostrum, as soon as possible, is so important.

1. Physiology and neonatal survival

At birth, ambient temperatures can drop from 39°C in utero to 10°C or lower. Maintenance of body temperature depends on the balance between heat loss and heat production. Heat loss is mainly affected by body surface area (small lambs have a higher surface area/BW ratio than large lambs, thus they lose heat faster and are more at risk of hypothermia) and the insulation value of the coat (short, fine birth coats have lower insulation value than long, coarse coats). A wet coat, by amniotic fluid, reduces insulation value but removal of the fluid by maternal licking or grooming contributes to the lamb's ability to maintain normal body temperature. Within 10 min of birth, the lamb increases heat production but important inter-individual variations are observed (Dwyer and Morgan, 2006), and in the most extreme cases, where lambs are unable to generate sufficient heat, hypothermia is irreversible. Heat production comes from two main mechanisms in the neonate: non-shivering thermogenesis, mostly if not all attributable to brown adipose tissue, and shivering thermogenesis, which usually takes place under cold conditions, mainly in older, dry lambs, and metabolizes muscle glycogen.

Colostrum contains nutrients, immunoglobulins, and other elements such as enzymes, hormones, growth factors, and neuroendocrine peptides. Colostrum contains fat (7% to 13%), non immunoglobulin protein (4% to 10%), and lactose (2% to 5%), and provides 6 to 7 kJ of energy/ml (Nowak and Poindron, 2006; Banchero et al., 2015). Early ingestion of colostrum has an additional benefit in that it increases heat production by 17% even if body energy reserves are still replete, enhancing resistance to hypothermia. Under optimal conditions, neonates would consume sufficient amount of colostrum to thermoregulate and acquire energy, delayed suckling may lead to energy reserves being exhausted within 6 h of birth and result in depressed heat production. Insufficient intake of colostrum is the second major factor affecting neonatal survival after body reserve depletion (Dwyer et al.,2015).

Between birth and 24 h post-partum, lambs are at a higher risk of hypothermia and require an adequate intake of colostrum to ensure sufficient energy reserves. Increased colostrum ingestion has been shown to increase rectal temperature and circulating glucose concentration (Plush et al., 2016). The amount of colostrum needed by a lamb depends on the energy required for heat production and thermoregulation.

During the first 24 h of life, an indoor-housed lamb requires on average 150 g of colostrum per kg of body weight when temperatures vary between 2 and 10 ◦C (Pattinson et al., 1995). However, when a lamb is wet and the external environment is rainy and windy, colostrum requirements increase by 150% (McCance and Alexander, 1959). For example, when starved and non-starved newborn lambs were exposed to

cold environmental conditions (9 ◦C), the starved lambs expended more energy than non-starved lambs indicating that lambs that consume sufficient colostrum had enough energy reserves to offset the high energy loss from cold exposure (Alexander, 1962). Once environmental challenges are overcome, colostrum plays a role in mediating the ewe-lamb bond and immune system development.

2. Colostrum production and composition

Colostrum production, called colostrogenesis, begins in late gestation and is the first food and source of nutrition for the newborn lamb/kid (Castro et al., 2011; Agenbag et al., 2021). Herd management, gestation length, parity, breed, and nutrition regimen, as well as the number of offspring and birth weight, have the potential to influence colostrum quantity and quality (Boland et al., 2005a, 2006; Castro et al., 2011; Campion et al., 2019). Compared to regular milk, protein, fat, mineral, and vitamin concentrations are greater in the colostrum. Other components like leukocytes, hormones, antimicrobials, anti-inflammatories, and growth factors, such as IGF-1, are also increased in colostrum, helping intestinal development (Castro et al., 2011; Hernández-Castellano et al., 2014; Akers, 2016).

3. Colostrum Quality and Management

The 3 pillars of colostrum management are: 1) adequate immunoglobulin G (IgG) concentration in colostrum (quality); 2) adequate quantity of colostrum (Alves et al., 2015); 3) timing of consumption (Dwyer et al., 2016).

Feeding good quality (50 g/L of IgG) and quantity colostrum to newborn kids and lambs during their first hours of life will be directly associated with their survival and will help decrease disease susceptibility during the preweaning period (Polidori et al., 2022), especially nowadays that the number of high-producing farms increased and in some cases lambs/kids are reared artificially, but still have high mortality rate in this category (Nowak and Poindron, 2006; Campion et al., 2019). A newborn lamb/kid requires from 150 to 290 ml of colostrum per kg of body weight (Mellor and Cockburn, 1986; Mellor and Murray, 1986; Pattinson et al., 1995; McGovern et al., 2015), ideally in average, a lamb/kid should receive 200 mL of colostrum per Kg of BW. This means that a 4 kg lamb must receive the minimum amount of 600 ml of good-quality colostrum during the first 18 hours after birth (Boland et al., 2005a; b, 2006), and ideally consume 800mL of good-quality colostrum for the first 24h. Another way to provide good quality would be to measure by IgG content and provide 8 g of immunoglobulins per body weight divided into three meals, i.e., a 4 kg lamb would receive a minimum of 32 g of IgG in the first day of life (Hernández-Castellano et al., 2015), as better results are found when the intake higher than 30 g of IgG in the first day of life. Additionally, these first meals, with high energy content provided in the colostrum, will help the offspring to thermoregulate, preventing starvation/hypothermia syndrome (Agenbag et al., 2021).

Colostrum IgG concentrations range widely between individual animals (Kessler et al., 2019). The relationship between colostrum and lamb serum IgG concentration has not been well defined, however, lambs from ewes with low pre-suckle colostrum IgG concentrations have been reported to have lower serum IgG concentrations (McGuire et al., 1983). Lamb/kid serum IgG concentrations are maximal 24–48 h after birth (Hernandez-Castellano ´ et al., 2015). No universally accepted critical value of serum IgG concentration exists to characterize the 'failure of transfer of passive immunity' (FTPI)

in lambs and kids, however, the threshold value of 15 g/L of serum has been widely adopted (Alves et al., 2015; Hunter et al., 1977; Turquino et al., 2011), with the assumption that passive transfer of immunity is not successful below this value.
Pointing this, sometimes the mother will not provide sufficient colostrum, or its quality will be questionable. It is important to mention that farms that work during lambing/kidding season, sometimes will not have personnel or time to assist and verify if all newborns have ingested the necessary volume of colostrum during the first hours after birth. Also, when having multiple offspring, the mother could probably not have sufficient colostrum volume to provide the ideal amount of IgG to their progeny (Polidori et al., 2022). Thus, having a protocol that all newborns are bottle-fed or tubed with fresh, thawed, or dried colostrum will help ensure good passive immunity transfer and avoid starvation/hypothermia syndrome. Additionally, herds with high lentivirus prevalence such as caprine arthritis-encephalitis or Maedi Visna could benefit from using pasteurized or dried commercial colostrum, since these diseases have high transmission via colostrum (Kaba et al., 2022).

4. Colostrum supplementation

The use of colostrum powder, as a supplement, is recommended when dam colostrum is not available, herd management and labor to follow lambs and kids are limited, high presence of pathogens transmitted via colostrum, or also to provide extra supplementation to the newborns since is convenient to store, mix and feed. Bovine colostrum showed that this could be a valid source to provide IgG uptake to the blood, promote good passive immunity, and improve the metabolic status of small ruminant newborns (Zhu et al., 2021). It is also tested for prevalent diseases and contains antibodies against most of the microorganisms that infect kids and lambs.
Providing mother's, pasteurized or dried colostrum as soon as possible is one of the most important managements to due. Knowing this is important to mention that there is no difference in providing colostrum using a nipple bottle or esophageal tube (Desjardins-Morrissette et al., 2018), highlighting that colostrum is the only feed that can be fed via an esophageal tube in the first day of life.
Additionally, since small ruminants' newborn's digestive tract is proportionally smaller than calves' digestive tract, it is recommended three feeding regimens totaling 15% of the body weight. It is important to highlight that if is been used a colostrum powder as a replacement, it should be reconstituted in water and not in milk. Maternal colostrum, when failing in quality <22%, can also be enhanced with colostrum powder supplement, bringing its quality up to the excellent threshold (>26% Brix).
Therefore, in summary, colostrum should be fed at 38 - 39oC, in three meals from six to eight hours apart, whereas the first meal is given a maximum of one hour after birth, with an average amount of 150-200 ml/ kg, and total amount of 30 g or more of IgG in the first day (McGovern et al., 2015; Godden et al., 2019). To quantify the success of this protocol passive immunity can be measured in small ruminants, and is defined as serum IgG concentration > 15 mg/ml (Alves et al., 2015; Zhu et al., 2021), or serum total protein concentration >5.3g/dL (Massimini et al., 2006).

5. Conclusion:

The critical role of birthweight as a risk factor for neonatal mortality has been emphasized throughout: lamb vigour, udder-seeking and thermoregulatory ability are

impaired in low birthweight lambs, and heavy lambs are vulnerable to dystocia and birth injury. Thus, ensuring optimal colostrum management would help increase lamb/kid vigour, and would guarantee the energy required to thermoregulate and survive, increasing the glucose status of the newborn.

Also to increase the neonate's ability to survive, access to colostrum during the neonatal period has the potential to improve the future production, development, and reproductivity efficiency.

An adequate intake of colostrum is a fundamental component of lamb survival. Many of the risk factors for lamb mortality have their root in preventing or reducing the uptake of sufficient colostrum by the lamb. Ensuring that neonatal ruminants gain access to and ingest colostrum as soon as possible after birth is probably the single most important factor in their subsequent survival. Therefore, some newborns may require the provision of supplementary colostrum.

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Methane Mitigation Feed Technologies

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It has been well-established that methane emissions from cattle are part of the biogenic carbon cycle and thus have a limited impact on the climate as compared to burning of fossil fuels. While it has been categorically proven that cattle are not the problem, cattle can be part of the solution to climate change.

At present, methane mitigation strategies for cattle are primarily feed additive solutions that can be delivered as part of a total mixed ration and are most suited for cattle with ad libitum access to total mixed rations such as feedlots or dairies. The most successful mitigants are part of a class of molecules that competitively block co-enzyme attachment in key steps of the Wolfe cycle, a pathway of methanogenesis. The two primary molecules in this class are 3- nitrooxypropanol and bromoform.

The mechanism of action of 3-nitrooxypropanol will be discussed. Practical discussions regarding formulations and delivery of this additive will be examined. The results of a recent large-scale commercial feeding trial of 3-nitrooxypropanol in Australia will be shared. A total of 9492 crossbred steers and heifers (body weight 362.8kg ± SD 30.4 kg) were randomly assigned to one of two treatments (n=237/treatment, single-sex): control (no 3-NOP) or 3-NOP (100 mg/kg DM dose of 3- NOP) and fed for the 106d finishing period. A summary of all recent publications on feeding 3- nitrooxypropanol in feedlot cattle globally will be shared.

The mechanism of action of the asparagopsis derived bromoform will be discussed. Results of the most thorough evaluation of feeding bromoform in feedlot cattle to date will be discussed. A single blinded randomized complete block design was conducted to evaluate the effect of SEAFEED™, a bromoform containing canola oil on emissions, performance, animal health, and carcass characteristics of Angus feedlot cattle. The results of this trial answer the critical questions of the impact of bromoform on daily emissions, average daily gain, daily feed intake, animal health, carcase characteristics, sensory testing of beef, rumen health, and residue testing for bromoform, bromine, and iodine in kidney, fat, and muscle. The results of this trial demonstrate the mitigant power of bromoform and the suitability of this product in feedlot cattle. Important feeding strategies including formulation and delivery of this product will be discussed.

Methane production, methane yield, and methane intensity will be discussed and compared. The mitigation effect of alternative products such as essential oils, ionophores, and more novel solutions such as hydrogen sinks and vaccine technologies will be discussed. An update regarding current global research on methane mitigation solutions for grazing cattle such as lick blocks, water supplementation, and rumen delivery systems will be examined. This presentation serves as an update of the current state of research for methane mitigation technologies in cattle.

Nutritional management of transition dairy cows

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Physiological, Hormonal, Metabolic, Immune and Ruminal Changes Feeding and management in transition period can have a substantial effect on herd's health, productivity and ultimately sustainability. The transition period in dairy cows is generally defined as the last 3 weeks of pregnancy and the first 3 weeks of lactation. It is well known that rapid fetal growth, mammary gland growth and development, colostrum synthesis and dramatic changes in the endocrine status occur during the prepartum period. Those hormonal changes during prepartum period are to prepare the cow for increasing in energy demands after the parturition (Ehrhardt et al., 2016). Plasma insulin, insulin-like growth factor 1 (IGF1), and leptin level decrease and growth hormone increases as the cow progresses from late gestation to early lactation, with acute changes in plasma concentrations at parturition (Kunz et al., 1985; Doepel ct al., 2002; Rhoads et al., 2004). Insulin-sensitive tissues in periparturient cows display insulin resistance so that glucose can be directed to the developing fetus and to the mammary gland (NASEM, 2021).

The changes in endocrine and metabolic status and the decrease in dry matter intake (DMI) that usually occurs around parturition influence metabolism and lead to mobilization of fat from adipose tissue and glycogen from the liver. In healthy dairy cows, from about 2 weeks prepartum until 3 days prepartum, plasma non esterified fatty acids increase from <0.2 mEq/L to about 0.3 mEq/L, and then 1 or 2 days before calving, concentrations increase abruptly and usually reach their peak by the second or third day of lactation (often between 0,8 and 1.0 mEq/L) (NASEM, 2021). Concentrations then decrease slowly over the next 2 to 3 weeks (Doepel et al., 2002; LeBlanc et al., 2005). In cows that develop health disorders such as a displaced abomasum, prepartum plasma NEFA concentrations often exceed 0.5 mEq/L and can exceed 1.0 mEq/L during the immediate postpartum period (LeBlanc et al., 2005). High rates of lipolysis and the subsequent elevated NEFA are associated with inflammation and immune system dysfunction (Bradford et al., 2015; Contreras et al., 2018) and can be a risk factor for ketosis.

Because of decresing the DMI relative to milk production in the fresh period (Gencoglu et al., 2019a), cows can mobilize substantial amounts of body protein (Bell et al., 2000). Plasma levels of 3-methyl histidine (a marker of protein breakdown) can be high the first few weeks of lactation (van der Drift et al., 2012). Estimates of the amount of body protein mobilized during fresh period vary widely, Tebbe and Weiss (2021) show that by about 4 weeks of lactation, body protein mobilization ceases.

During the periparturient period, the rumen wall changes in size, morphology, and functionality. Rumen tissue mass increased about 5 percent between 1 week prepartum and 10 days postpartum and continued to increase through at least 120 days postcalving

(Reynolds et al., 2004). These changes indicate increasing absorptive capacity within the rumen tissue during the fresh period.

Around calving period, cows experience varying degrees of immunosuppression (; Hansen, 2013). The neutrophil and lymphocyte function is decreased (Rinaldi et al.,2008), levels of immunoglobulins are reduced at last part of gestation because of transfer to colostrum (Her et al., 2011). Estrogen, progesterone, and cortisol concentrations are high before calving and can suppress immune function. Nutrient consumption is decreaced around parturition, which may also contribute to immunosuppression. Because Ca is integral to immune cell activation (Kimura et al., 2006), hypocalcemia increases the degree of immuno-suppression (Martinez et al., 2018). Decreases in some of those nutrients may contribute to oxidative stress, which can also contribute to immunosuppression (Sordillo,2013).

Fatty Liver

When blood concentrations of NEFA are increased, fat accumulation in the liver occurs when uptake of fatty acids exceeds the capacity of the liver to oxidize or secrete (Grummer, 2008). Ketosis almost always occurs when cows have moderate (5 to 10 percent of liver wet weight as triacylglyceride) to severe (greater than 10 percent fat) fatty liver. Because most cows have increased NEFA during the peripartum period, fresh cows mostly have some degree of hepatic fat accumulation (NASEM, 2021). Fatty liver is a major risk factor for displaced abomasum, ketosis, and immune dysfunction. A major risk factor for development of fatty liver is obesity (Bobe et al., 2004).
Supplementing rumen-protected choline during the peripartum period can reduce liver fat concentrations (Lima et al., 2012; Zenobi et al., 2018). Niacin has antilipolytic properties, but unless supplemented at very high rates, it usually does not affect plasma NEFA concentrations (Grummer, 2008). Supplementing peripartum cows with rumen-protected niacin has reduced plasma NEFA but has not markedly affected liver lipid concentrations (Yuan et al., 2012).

Ketosis

Excessive amounts of long-chain fatty acids are released in cows' severe negative energy balance after calving. Ketone bodies are end products of oxidation, and when these accumulate in the blood, clinical signs can be observed. Increased blood BHBA has been associated with increased risk of health problems, low milk yield, and decreased reproductive efficiency. A definitive diagnosis requires some measure of ketones in blood, urine, or milk. (Raboisson et al., 2014). The cutoff for separating healthy lactating cows from cows with subclinical ketosis has varied between approximately 1.0 and 1.4 mmol/L (Raboisson et al., 2014), but a value of >1.2 mmol/L of BHBA is commonly used to define subclinical ketosis.

Holtenius and Holtenius (1996) classified ketosis as type 1 or type 2. Type 1 ketosis generally occurs a few weeks after parturition when milk production and glucose need by the mammary gland are high and is usually not associated with excessive hepatic fat concentrations. Type 2 occurs at very near parturition and is usually associated with fatty liver. Type 1, blood glucose and insulin concentrations are lower and ketone concentrations are higher compared to healthy cows. Low insulin probably enhances

fatty acids oxidation by decreasing hepatocyte malonyl-CoA concentrations and sensitivity of carnitine palmitoyltransferase 1 to malonyl-CoA concentrations (Emery et al., 1992). Increasing dietary starch postpartum reduces blood BHBA and increases glucose (McCarthy et al., 2015), on the other hand, Gencoglu et al. (2019b) did not see any differences on BHBA levels in cows fed diet with amylase supplemantation.

Refained Placenta and Metritis

Retained placenta is defined as failure of the fetal membranes to be expelled within 24 hours alter parturition (Kelton et al., 1998). Metritis is defined as postpartum cows with abnormally enlarged uterus with fetid red-brown watery or purulent vaginal discharge within the first 21 days after calving with or without systemic signs of illness. Cows suffering from (60 to 80 percent) with retained placenta will have metritis, but the incidence of metritis is usually much greater than the incidence of retained placenta (Gilbert et al., 2005). Retained placenta and metritis impair the reproductive efficiency Multiple physiologic and nutritional factors have been implicated as causes of retained placenta and metritis. Dystocia, twining, stillbirth, and caesarcan section increase the risk for retained placenta (Han and Kim, 2005). Older cows generally are at greater risk than first-parity cows, and a short gestation period increases risk of retained placenta (Han and Kim, 2005).

Most studies evaluating nutritional influences on retained placenta evaluate supplementation during the entire dry period or during the last 2 or 3 weeks of gestation. Inadequate supply of selenium (Se), vitamin E, vitamin A, and ß-carotene is related to increased prevalence of retained placenta.

Hypocalcemia

Plasma concentrations of Ca should be 9 to 10 mg/dL. However, an acute and severe form of hypocalcemia known as milk fever occurs in nearly 5 percent of multiparous dairy cows as a result of the large and sudden secretion of Ca in milk that occurs at the onset of lactation (NAHMS USDA: 102). Cows with plasma concentrations less than 8.0 (Reinhardt et al., 2011) to 8.6 mg/d alarine, et al., 2012) are considered subclinically hypocalcemic. Cows with subclinical hypocalcemia are at increased risk for immune dysfunction, metritis, displacement of the abomasum, retained placenta, mastitis, and ketosis (Kimura et al., 2006; Martinez et al., 2012).

Ca homeostasis is mediated primarily by the parathyroid gland, which secretes parathyroid hormone (PTH) in response to any reduction in blood Ca concentration. The PTH stimulates release of Ca from bone stores, reduces the amount of Ca lost via urine, and activates the renal enzyme that produces the vitamin D hormone, 1,25-dihydroxyvitamin D. That hormone stimulates transcellular absorption of Ca across the intestinal epithelium to greatly increase uptake of dietary Ca (Goff, 2018).

Heifers rarely develop clinical milk fever, although 25 percent may experience subclinical hypocalcemia. The incidence of clinical and subclinical hypocalcemia increases with each subsequent lactation (Reinhardt et al., 2011).

Adding Cl- and SO4- without Na or K to the precalving diet can greatly reduce the degree of hypocalcemia at calving. Dietary Cl is absorbed with nearly 100 percent efficiency. Sulfate anions can also acidify the blood but, because of lower absorption SO4-, has just 60 percent of the acidifying activity of Cl (Goff et al., 2004). Blood pH is difficult and expensive to measure, but urine pH generally shows blood pH and can be measured on farm to determine the level of metabolic acidosis experienced by the cow. Diets that reduce urine pH values below 7.0 and usually closer to 6.0 generally improve Ca status (Charbonneau et al., 2006). As DCAD decreases, the degree of hypocalcemia will also generally decrease (Charbonneau et al.,2006).

The strategy involves limiting absorbed Ca so that the cow is in negative Ca balance for at least 7 to 14 days before calving. Negative Ca balance stimulates secretion of PTH within 3 to 4 days of the dietary Ca reduction, and PTH concentrations will remain elevated until after calving (Goings et al., 1974).

Based on current Ca requirements and an availability coefficient of 0.44 for a high-forage diet, to meet the needs of a late gestation 650-kg cow, the diet would have to contain about 52 g of Ca or 0.43 percent Ca (based on an intake of 12 kg) (NASEM, 2021).

Hypomagnesemia can contribute to hypocalcemia and the blood Mg levels is normally 1.9 to 2.4 mg/dL (0.8 to 1 mmol/L.), but if blood Mg concentration falls below 1.25 mg/dL (0.5 mmol/L), the ability of the parathyroid gland to secrete PTH is compromised and blood Ca concentration rapidly decreases (Littledike and Goff, 1987). Based on a meta-analysis, an increase in dietary Mg concentration from 0.3 to 0.4 percent of DM, while maintaining DCAD and Ca constant, could result in an approximate 62 percent decrease in milk fever risk (Lean et al., 2006).

Displaced Abomasum

Displacement of the abomasum (DA) is a costly multifactorial disorder. The transition period and several weeks subsequent to calving is the major risk period for development of displaced abomasum (Stengärde et al., 2010).

Decreased DMI around parturition is likely a cause of DA, although direct evidence is limited. Shaver (1997) outlined several factors that can increase the risk of DA and also likely limit or reduce DMI. These include limited feed availability, crowded pens and feed bunks, improperly mixed total mixed ration (TMR), sorting, and inclusion of unpalatable ingredients in the diet, among other factors.

Summary

· Meet energy needs through appropriately well balanced energy and fiber content to control intake;

· Meet the metabolic protein (MP) at least 1100 g is consumed for all cows, should delivery of 1300 g MP to account for DMI variation

· Additional grain in close-up diet

· Maintain calcium homeostasis macro mineral content, especially relationships between potassium and magnesium and calcium

· Sufficient dietary vitamins and trace minerals from available sources to meet needs of cow, colostrums, and fetus to minimize negative effects on immune response

· Manage animal grouping strategies and dietary formulations in late lactation and dry period to achieve proper BCS and minimize the number of excessively fat or thin cows
· Minimize cow stress response by ensuring adequate cow comfort
· Proper stall resting area
· Sufficient feed bunk and watering space
· Heat stress and good ventilation
· Provide clean, dry bedding of sufficient cushion
· Minimize pen moves resulting in social upheavals
· Minimize overcrowding in critical groups

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Reproduction

Applying assisted reproductive technology and reproductive management to reduce CO2-equivalent emissions in dairy and beef cattle

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Abstract

Methane emission from beef and dairy cattle combined contributes around 4.5-5.0% of total anthropogenic global methane. In addition to enteric methane (CH4) produced by the rumen, cattle production also contributes carbon dioxide (CO2) (feed), nitrous oxide (N2O) (feed production, manure) and other CH4 (manure) to the total greenhouse gas (GHG) budget of beef and dairy production systems. The relative contribution in standard dairy systems is typically enteric CH4 58%, feed 29% and manure 10%. Herds with low production efficiency can have an enteric CH4 contribution up to 90%. Digestibility of feed can impact CH4 emission intensity. Low fertility herds also have a greater enteric CH4 contribution. Animals with good feed conversion efficiency have a lower emission intensity of CH4/kg of meat or milk. Feed efficient heifers tend to be lean and have delayed puberty. Fertility is a major driver of profit in both beef and dairy cattle, and it is highly important to apply multi-trait selection when shifting herds towards improved efficiency and reduced CH4. Single nucleotide polymorphisms (SNPs) have been identified for feed efficiency in cattle and are used in genomic selection. SNPs can be utilized in artificial insemination and embryo transfer to increase the proportion of cattle that have the attributes of efficiency, fertility and reduced enteric CH4. Prepubertal heifers genomically selected for favorable traits can have oocytes recovered to produce IVF embryos. Reproductive technology is predicted to be increasingly adopted to reduce generation interval and accelerate the rate of genetic gain for efficiency, fertility and low CH4 in cattle. The relatively high contribution of cattle to anthropogenic global methane has focused attention on strategies to reduce enteric CH4 without compromising efficiency and fertility. Assisted reproductive technology has an important role in achieving the goal of multiplying and distributing cattle that have good efficiency, fertility and low CH4.

Keywords: cattle, enteric methane, efficiency, fertility, assisted reproductive technology

Introduction

The global population of beef and dairy cattle combined is approximately 1.5 billion. Amongst domestic herbivores globally, cattle contribute about 20% of meat and 85% of milk. The global demand for meat and milk is projected to increase by 57% and 48%,

respectively, between 2005 and 2050 (Alexandratos & Bruinsma, 2012). Cattle, therefore, will continue to have a very important role in future global food security (Davis & White, 2020). Beef and dairy production occur across diverse environments and in both extensive and intensive systems (Faverdin et al., 2022). Extensive cattle grazing is found in rangelands and savannas that are suited to low-input and low-cost animal production. Intensive beef and dairy systems utilize, and add value to, feed sources that are either unsuitable or surplus to human consumption. Grazing lands cover about 25% of the global landmass (Mottet et al., 2018) and intensive beef accounts for <15% of global
beef production (Mottet et al., 2017). The environmental footprint of cattle production has received increased attention globally (Knapp et al., 2014; Faverdin et al., 2022). Methane emission from cattle has been recognized for around 30 years (Johnson & Johnson, 1995) and has become a particular focus as cattle contribute around 4.5-5.0% of total anthropogenic global methane (Wallace et al., 2015; Hayes et al., 2016; de Haas et al., 2021; Faverdin et al., 2022; Hossein
Zadeh, 2022; Galyean & Hales, 2023). Most methane produced by cattle is from enteric fermentation of complex carbohydrates into simple sugars by methanogenic protozoa (Bowen et al., 2020). The biology and function of the rumen has been well reviewed (Ross et al., 2013; Knapp et al., 2014). The ability to digest cellulolytic material into usable energy and protein is arguably the greatest advantage but also the greatest disadvantage of cattle. The relative abundance of ruminal methanogenic and non-methanogenic microbes influences the amount of methane produced by an individual animal (Bowen et al., 2020). The population of ruminal microbes can now be determined by microbial gene abundance (Roehe et al., 2016).

Assisted reproductive technologies can have a major impact on improving productivity in beef and dairy cattle. Artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) increase the rate of dissemination of animals with traits that have high genetic merit and high productive capacity. However, the mature technologies of AI and MOET do not increase the rate of genetic gain from one generation to the next. The latter is controlled by generation interval which is relatively long in cattle (Schefers and Weige 2012; Kasinathan et al. 2015). Generation interval can be shortened in cattle by utilizing oocytes from heifers early in life to produce IVF embryos (Baruselli et al., 2016; Baldassarre & Bordignon, 2018). This review seeks to demonstrate how assisted reproductive technology (ART) and reproductive management can be used to generate cattle that have improved efficiency and produce less methane.

Reproductive efficiency in cattle and application of artificial insemination to improve efficiency and reduce methane emission

In beef cattle, the cow-calf unit utilizes approximately 70% of resources. Selection for reproductive efficiency therefore has a major bearing on both efficiency and profitability. With high reproductive efficiency, fewer cows are required to produce the next generation of calves, and this reduces resource requirement, herd methane production, and costs (Hegarty and McEwan, 2010). Also, reproductively inefficient cows are removed from herds. In a United States beef production system, an improvement in reproductive efficiency (0.5 to 1 calves/year) resulted in a

34% reduction in water use, 44% reduction in land use, and 39% reduction in the CO2-equivalent (CO2-eq) footprint (Davis and White, 2020). ART can be incorporated into beef breeding programs to further improve efficiency and reduce CO2-eq emission intensity. In Brazil, the use of timed artificial insemination (TAI) in a breeding herd reduced age at first calving from 48 to 24 months and increased weaning rate from 60% to 80% compared with natural mating (Abreu et al., 2022). There was a 37.7% reduction in pasture required and 85.4% reduction in CO2-eq to produce 400 calves (Abreu et al., 2022). The CO2-eq was calculated according to livestock units (1 LU=450 kg of live weight) and a stocking rate of 1 LU per hectare of pasture was estimated to produce calves (Figueiredo et al., 2017). The low reproductive efficiency system (natural mating) emitted 3,714.5 tons of CO2-eq per year while the high reproductive efficiency system (TAI) emitted 2,311.3 tons of CO2-eq annually. The TAI system generated US$84,196 in credit for reducing CO2-eq emissions (quoted at US$60 per 1-ton CO2-eq). TAI has been applied in beef heifers to reduce age at first pregnancy and calving (Baruselli et al., 2017) which impacts lifetime reproductive efficiency and CO2-eq emissions. TAI can also be utilized to manage inter-calving intervals so that cows produce a calf annually (Sá Filho et al., 2013; Baruselli et al., 2018a).

The same basic principles addressed above apply in dairy cattle (Hutchinson et al., 2013). For example, lowering the age at first calving and culling frequency reduced the number of
replacement heifers needed and enteric methane emission per unit of kg energy-corrected milk (CH4/ECM; Knapp et al., 2014). Improving the fertility of dairy herds can potentially reduce methane emission by up to 25% (Garnsworthy, 2004). We recently studied the influence of calving interval (CI, i.e. reproductive efficiency) on the CO2-eq footprint of lactating dairy cows using life cycle assessment methodology (Abreu et al., 2023). A comparison was made between production and CO2-eq/milk (corrected for fat and protein content) of cows with a CI of 13 or 15 months. The lactation period was estimated at 11 and 13 months for cows with a CI of 13 or 15 months, respectively (Cole & Null, 2009; Biassus et al., 2010). Total greenhouse gas emissions for 1 kg of milk (CO2-eq/milk) was 0.657 when the CI index was 13 months and 0.703 (7% increase) when the CI index was 15 months.

Embryo technology to mitigate methane emission

Dairy cattle can suffer heat stress (HS) during summer which decreases dry matter intake (DMI), daily gain, milk yield, and fertility (Kadzere et al., 2002; Hansen, 2007). During HS, milk production decreases more than dry matter intake which increases the CO2-eq emission/kg energy corrected milk (Rhoads et al., 2009). HS contributes to culling and death of cows (St-Pierre et al., 2003). The reduction in fertility is associated with altered ovarian folliculogenesis and oviductal function and increased embryonic mortality. The latter can be managed during periods of HS by replacing natural mating and artificial insemination (AI) with the transfer of either in vivo or in vitro derived embryos to cows on day 7 of the estrous cycle (Hansen, 2007; Baruselli et al., 2020).

We developed a simulation model which compared the use of AI or embryo transfer (ET) in HS dairy cows. The model assumed that pregnancy per AI (P/AI) and P/ET during HS

were around 17 and 40%, respectively, and the service rate was 60% for AI and 50% for ET (ET was performed only on animals with a corpus luteum) (Baruselli et al., 2018b). The pregnancy rate following 105 days of breeding was 34.6% for AI and 53.1% for ET (53.6% increase). Cows subjected to AI had a greater number of days open (59.3 days) than cows exposed to ET (52.5 days) after the beginning of the breeding program. This shows that it is possible to increase the 21-day pregnancy rate by eight percentage points using ET in place of AI in HS dairy cows. As noted earlier, shorter inter-calving intervals are associated with a reduced CO2-eq budget in cattle.

As noted earlier in this review, the mature technologies of AI and MOET do not increase the rate of genetic gain. The latter is controlled by generation interval which is relatively long in cattle (Schefers & Weige, 2012; Kasinathan et al., 2015). Generation interval can be shortened in cattle by utilizing oocytes from heifers early in life. Waves of follicular growth occur before birth and in the first weeks after birth in heifers (Evans et al., 1994a, 1994b; Monteiro et al., 2009). Oocytes can be recovered before birth (velogenesis; Betteridge et al., 1989; Georges & Massey, 1991; Kauffold et al., 2005) and well before puberty (Onuma et al., 1970; Baruselli et al., 2016), and used to generate viable embryos in the laboratory using in vitro embryo production (IVEP) (Baruselli et al., 2022). Prepubertal heifers show a good ovarian follicular response to FSH superstimulation and a relatively large number of oocytes can be retrieved for IVEP (Baruselli et al., 2022). IVEP is less efficient for oocytes from young heifers compared with mature heifers and cows and further research is needed to optimize IVEP in prepubertal heifers (Baruselli et al., 2022). Notwithstanding, IVEP with oocytes from young heifers has emerged as a fundamental enabling technology for the exploitation of genomic selection to produce cattle defined by efficiency, fertility and low CH4 emission.

Balancing feed efficiency in meat and milk production with fertility and low CO2-eq emission

Cattle consume a relatively large amount of biomass and have a low feed conversion ratio compared with other livestock (FAO, 2018; Mottet et al., 2018). The provision of feed typically accounts for 70-80% of production costs in both extensive and intensive systems (Mottet et al., 2018). There is considerable interest, therefore, in identifying and multiplying cattle that have improved feed efficiency (Løvendahl et al., 2018; Davis and White, 2020). This applies to both extensive and intensive systems (Hietala & Juga, 2017; Kava et al., 2023). Associations between feed efficiency, methane production, and sustainability, have been known for more than 20 years (Arthur & Herd, 2005; Nkrumah et al., 2006; Freetly & Brown-Brandl, 2013). The relatively high heritability of growth and feed efficiency in cattle was recognized some 70 years ago and subsequently confirmed (Knapp Jr & Nordskog, 1946; Berry & Crowley, 2013; Gonzalez-Recio et al., 2014; Sypniewski et al., 2021).

More recently, single nucleotide polymorphisms (SNPs) have been identified for feed efficiency in cattle and have been used in genomic selection (Arthur, 2015; Sypniewski et al., 2021; Madilindi et al., 2022; Buss et al., 2023). As noted earlier, the relative abundance of ruminal acetogenic and methanogenic microbes influences methane emission by individual animals. There is a significant host effect on the ruminal microbe population, and it has been proposed that microbial gene abundance can be used to select cattle for feed efficiency and growth (Roehe et al., 2016). The genome of cattle

can influence the population of ruminal microbes and hence the ruminal microbe genome profile which determines methane production (Difford et al., 2018; O'Hara et al., 2020; Gonzalez-Recio et al., 2023). Characterization of the ruminal microbe gene profile has been proposed as an alternative to expensive, time consuming methods for measuring feed efficiency in individual cattle (Arthur & Herd, 2005; Basarab et al., 2013; Kenny et al., 2018; Terry et al., 2021).

Growth and feed efficiency genes show single nucleotide polymorphism (Abo-Ismail et al., 2013; Madilindi et al., 2022; Buss et al., 2023). Methane emission also shows single nucleotide polymorphism in cattle (Sarghale et al., 2020). The advent of molecular gene markers has created the opportunity to accurately identify cattle with desirable genes and to then use ART to rapidly multiply and disseminate cattle with improved feed efficiency and growth performance. Efficient cattle were reported in one study to have reduced CH4 (g/day) and CO2-equivalent (g/day) emissions (Callegaro et al., 2022). The breeding technology used to generate efficient cattle will be governed by the production system and resources available. For example, AI and ET are already utilized in intensive dairy systems. Artificial insemination can be adopted in extensive beef systems as demonstrated in Latin America (Baruselli et al., 2004; Ferraz et al., 2012; Sartori et al., 2016; Mapletoft et al., 2018; Bó et al., 2018). Low-input, low-cost beef systems (North and South America, northern Australia, South Asia, Sub-Saharan Africa) will continue to rely on natural mating. For these regions, central breeding facilities will utilize genomic selection and ART to produce male embryos and/or bulls for dissemination for natural mating.

Whilst feed efficiency is undoubtedly a commercially important trait in beef and dairy cattle, selection for feed efficiency should not be at the expense of other important traits (Mu et al., 2016). As this review has argued, fertility has a major impact on enterprise productivity and profit in both beef and dairy systems. Studies in young growing British and European (Bos taurus) bulls consistently showed negative associations between feed efficiency and fertility measures including testicular growth and morphology and the characteristics of seminal plasma and spermatozoa (Awda et al., 2013; Fontoura et al., 2016; Montanholi et al., 2016; Bourgon et al., 2018). In contrast, a study in growing composite bulls (Bos taurus x Bos indicus) found that fertility measures did not differ for bulls of different feed efficiency (Kowalski et al., 2017).

Heifers with improved feed efficiency were reported to be leaner and reached puberty later than heifers with lesser feed efficiency (Randel & Welsh, 2013). In another study, heifers with good feed efficiency attained puberty earlier than heifers with poorer feed efficiency (Canal et al., 2020). Other studies in female cattle have also shown either a negative effect of feed efficiency on fertility (Mu et al., 2016; Ferreira Jr et al., 2018) or no effect (Crowley et al., 2011; Davis et al., 2016). A study in dairy cows under commercial conditions reported that cows with high feed efficiency had a greater inter-calving interval (Vallimont et al., 2013). Dairy cattle selected for milk yield and feed efficiency had a reduced methane budget resulting from increased milk yield (Knapp et al. 2014). The impact of this selection strategy in an intensive dairy system was estimated to be a reduction of 9-19% in CO2-eq emission/kg energy-corrected milk (Knapp et al. 2014). In another study in dairy cows, selection based on genetic potential for milk production was associated with a decline in fertility, an increase in non-

productive cows, and overall increase in CO2-eq emission for the production system (O'Brien et al., 2010). Another study in dairy cows reported low genetic correlations between methane production and fertility traits (Zetouni et al., 2018). Given the contrasting reports there is a need for further studies on feed efficiency, methane production, and lifetime fertility in cattle. The above studies have also demonstrated the importance of multi-trait selection in cattle breeding programs and the need to balance feed efficiency with other commercially important traits, in particular fertility (Bonamy et al., 2019).

Enteric methane in production system life cycle assessment

Enteric methane forms part of the broader greenhouse gas (GHG) budget of beef and dairy production systems (Ibidhi & Calsamiglia, 2020). The broader GHG budget includes methane, nitrous oxide (N2O) and CO2 emission from manure, feed production, vehicles and transport, and other plant and equipment. The total GHG budget of a production system is determined by life cycle assessment (LCA) methodology standardized by ISO 14040 (ISO, 2006a) and ISO 14044 (ISO, 2006b) (de Vries et al., 2015; Kyttä et al., 2022). The relative contribution of different components of production systems to the GHG budget can vary greatly for different beef and dairy systems. One estimate for milk production was enteric methane 58.5% (CH4), feed production 29.4% (CO2, N2O) and manure 9.5% (CH4, N2O; FAO, 2018). The relative contribution of enteric methane can reach 91% in low efficiency systems (Chhabra et al., 2013). The digestibility of feed can also have a major impact on enteric methane contribution to the overall GHG budget (Pinares-Patiño et al., 2007; FAO, 2019; Eugéne et al., 2021). Herds with high fertility and high production efficiency have a reduced GHG budget (Strandén et al., 2022). In low fertility herds, replacement heifers can contribute up to 27% to the GHG budget (Garnsworthy, 2004). The contribution of replacement heifers decreases to 10-12% in high fertility herds. High fertility herds with fewer replacement heifers require less feed production and have reduced manure, which lowers methane and nitrous oxide emission.

Conclusions and future direction

The global attention on enteric CH4 production in cattle requires a response that involves collaboration between researchers and industry. Future generations of cattle will be characterized by better efficiency and fertility, which may reduce CH4 emission intensity. This will result from balanced multi-trait selection. There has been progress in the discovery of SNPs for efficiency and methane emission in cattle. These SNPs will be incorporated into assisted reproductive technology such as AI and ET for targeted multiplication and dispersal of cattle with defined production and environmental credentials. The urgency in moving to the next generation of cattle will see an increase in the production of embryos from genomically defined prepubertal heifers. This will reduce generation interval and accelerate the rate of genetic improvement to cattle defined by better efficiency and fertility and lower CH4 emission. The opportunity for cattle to be

a part of ecosystem management was recently highlighted (Thompson et al., 2023). The challenge remains to communicate the importance of cattle for food security and the environment (Manzano et al., 2023).

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Prohibition of estradiol for FTAI in cattle: Two worlds and One Health

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INTRODUCTION

The Americas account for 35% of the global bovine population, making it the region with the highest concentration of cows worldwide. The counties of this region produce more than 30% of the meat and 25% of the milk consumed worldwide. Five out of the top 10 countries that globally export beef are located in Latin America, highlighting the region's significant role in the beef industry. Livestock farming stands as a crucial sector for the economic and social development of these nations. In general, bovine production systems in Latin American countries are based on pasture and grazing throughout the year, strongly influenced by environmental conditions. Feeding and body condition varies throughout the year and reproductive efficiency is relatively low. In such conditions, Fixed-Timed Artificial Insemination (FTAI) represents a great technology that was introduced in the early years of this century. Nowadays, it is used massively and on a large scale, making a significant contribution to the improvement of livestock farming. To apply this technology in these countries, and also in some others, estradiol-based drugs (17β-estradiol and its esters) are routinely used. However, in the European Union (EU), the use of estradiol was banned in 2008, and since then, EU has been pressuring other countries that supply animal-origin foods to restrict their use as well. As a result, New Zealand banned the use of these drugs in 2008, and after few years in 2020, Uruguay became the first country in Latin America to do so. Currently, in Uruguay, the importation, production, commercialization, and possession of products containing 17 β-estradiol and its esters are not allowed. Following Uruguay, other countries in this region have begun to take restrictive measures for the use of estradiol and its esters, such as Paraguay and Argentina.

The One Health initiative for food production has become a paradigm that must also be considered in herd reproductive management. Estradiol has been considered by the EU as a threat to the safety of the meat or milk produced by cows that receive a dose of this drug. However, this argument lacks sufficient technical support, and the real cause of the restriction in FTAI protocols originates from commercial concerns between EU and North America. There is no evidence that the use of estradiol at the doses used in cows for FTAI and in embryo recipients generates safety concerns for consumers. Reading material specifically prepared for this topic is recommended (Menchaca, 2021). However, the decision to prohibit the use of estradiol in some regions has divided the FTAI's world into two: those countries that use estradiol and those that do not.

Considering the need to use alternatives to estradiol's esters in FTAI programs, this paper discusses different strategies to replace a) estradiol benzoate (EB) administered

at the beginning of the FTAI protocol as an inducer of follicular turnover, and b) estradiol cypionate (ECP) administered at the end of the protocol to induce ovulation.

ESTRADIOL/PROGESTERONE-BASED PROTOCOLS FOR FTAI

Pharmacological protocols based on estradiol and progesterone that control follicular development and ovulation for FTAI are widely validated and have been used for over 20 years in various conditions across all Latin American countries (Baruselli et al., 2018; Bó et al., 2018). This technology has shown a growing adoption rate by veterinarians and producers, representing a powerful tool to increase reproductive rates in cattle farming and introduce superior genetics through artificial insemination on a massive or large scale as was never possible before (Menchaca, 2021).

These protocols with estradiol and progesterone consist of inserting a progesterone-releasing intravaginal device for 7 to 8 days, along with the intramuscular administration of 2 mg of estradiol benzoate (EB) at the time of device placement. This treatment with both combined drugs inhibits the release of LH and FSH, inducing the atresia of the dominant follicle and synchronizing the emergence of a new follicular wave 3 or 4 days later (Bó et al., 1995). At the time of removing the progesterone device, a dose of prostaglandin F2α (PGF2α) to ensure luteolysis is administered along with a dose of equine chorionic gonadotropin (eCG), which promotes ovulatory follicular growth, ovulation rate, induces greater progesterone production in the first 15 days after ovulation, and increases pregnancy rates mainly in anestrous cows. A dose of estradiol cypionate (ECP) is administered as an ovulation inducer at the time of device removal. These treatments generally achieve an average of 50 to 60% pregnancy rate with consistent results when applied under appropriate conditions.

A recent modification made to these FTAI protocols has been the extension of the proestrus, meaning prolonging the interval between device removal and FTAI or ovulation. This advancement was based on several studies published in the 2000s and 2010s demonstrating that the period from intravaginal device removal to ovulation inducer administration (interval referred to as proestrus) affects pregnancy rate (Bridges et al., 2008). These results led to new TAI protocols, such as the 5-day Co-Synch (based on GnRH and progesterone) and the J-Synch (based on EB and progesterone), which under certain conditions improve pregnancy rate. In estradiol-based protocols, in J-Synch, EB was maintained at the beginning of the protocol, but to prolong proestrus, progesterone treatment was reduced to 6 days (instead of 7 or 8 days), and the administration of ECP at device removal was replaced by a dose of GnRH 60-72 h after device removal. By prolonging proestrus and delaying ovulation, the timing of FTAI is also delayed from 48-56 h to 60-72 h after device removal (de la Mata et al., 2018). This is the J-Synch protocol, which has been used in several studies in our region, demonstrating a significant improvement in pregnancy rate in heifers by 5 to 10 % (de la Mata et al., 2018; Núñez-Olivera et al., 2022). In recent years, this protocol has become one of the most widely used protocols in Latin America for inseminating heifers. However, in lactating cows, we have not found an improvement in pregnancy rate when

comparing this protocol with the conventional 7-8 day protocol with ECP, and for this reason, we continue to recommend the conventional protocol in cows with calves.

GnRH PROTOCOLS

In countries where estradiol salts are not available, protocols have been developed to synchronize ovulation through other strategies. The most emblematic protocol is Ovsynch, as it was the first FTAI treatment developed in the USA, a protocol that only involves the use of GnRH and PGF2α (Pursley et al., 1995). It involves administering a dose of GnRH to induce the LH peak, leading to ovulation of the dominant follicle and emergence of a new follicular wave 1 to 2 days later. Seven days later, a dose of PGF2α is administered to induce luteal regression, followed by a dose of GnRH 48 hours later, with insemination being performed 24 hours later (currently 12-16 hours later). This protocol works relatively well in cycling cows in good body condition but is not as effective in anestrous cows. One of the most significant improvements made to this protocol was the addition of progesterone or its analogs (Thompson et al., 1999; Lamb et al., 2001; Stevenson et al., 2003). Adding a progesterone-releasing device for 7 days from the administration of GnRH to the administration of PGF2α improves synchronization of the emerging follicular wave, oocyte quality, and the luteal response to PGF2α (Consentini et al., 2021). Another modification made to the Ovsynch protocol to simplify it was to administer GnRH and FTAI at the same time 48 hours after PGF2α, thus reducing the number of cow handling procedures prior to FTAI. With this modification, this protocol is known as Co-Synch (Geary and Whittier, 1998). Since then, this 7-day Co-Synch protocol, which does not include estradiol, has probably been the most widely used treatment in the USA for several years.

Some years later, other researchers proposed extending the proestrus of this Co-Synch protocol, shortening the progesterone treatment from 7 to 5 days and lengthening the interval from device removal to GnRH/FTAI administration from 48-56 hours to 72 hours (Bridges et al., 2008; 2014; Whittier et al., 2013; Day, 2015). With this new protocol known as 5-day Co-Synch, pregnancy rates superior to those obtained with the 7-day Co-Synch protocol were achieved (Day, 2015). By reducing the time of progesterone device treatment, the negative effect of persistent follicles in cows that fail to ovulate after the first GnRH is avoided (Day, 2015). Furthermore, prolonging proestrus allows for higher circulating estradiol concentrations before ovulation and higher progesterone concentrations in the subsequent luteal phase (Bridges et al., 2014), improving uterine environment and embryonic development (Bridges et al., 2008; Bridges et al., 2012). The 5-day Co-Synch protocol has been taken as a reference by our laboratory to be evaluated as an alternative to estradiol-based protocols under the most adverse production systems in Latin America. However, this protocol has some difficulties that must be considered and that we have been overcoming with different strategies presented in the following sections.

FIELD RESULTS IN PROTOCOLS EXCLUDING ESTRADIOL

Heifers

In our efforts to avoid the use of estradiol, we have evaluated various alternatives to achieve similar pregnancy rates while maintaining a field-friendly protocol with no more than three cattle handling sessions. The alternatives evaluated in our experiments have been based on the Co-Synch protocol, with some adaptations as described below.

In our evaluations involving heifers, we always maintained a control group using the J-Synch protocol, which includes estradiol at the beginning of treatment. This protocol has been noted for achieving the highest pregnancy rates in heifers and is widely used in Latin American countries. In our initial experiments, we explored the use of GnRH and injectable progesterone at the start of the protocol as alternatives to estradiol. The hypothesis behind the efficacy of injectable progesterone administration lies in its ability to suppress LH pulsatility, thereby favoring follicular turnover similar to what is achieved with estradiol at the protocol's initiation. In all protocols evaluated, we maintained the strategy of inducing a prolonged proestrus.

In our first experiment, we studied 1,947 Angus heifers across six replicates, with 1,463 heifers of 14 months and 484 of two years old. The majority of heifers of 14 months (59%) and two years (56%) had a detectable corpus luteum at the protocol's initiation. Within each replicate, heifers were divided into four experimental groups to receive: a) J-Synch protocol, consisting of an intramuscular injection of estradiol benzoate (2 mg) + intravaginal progesterone device for 6 days; b) GnRH-based protocol, with an intramuscular injection of GnRH (100 µg) + progesterone device for 5 days; c) injectable progesterone protocol, with an intramuscular injection of progesterone in oil (100 mg) + progesterone device for 6 days; d) control group, receiving no synchronization drugs, maintaining the progesterone device for 6 days. All groups received an intramuscular injection of eCG (200 IU) and a dose of PGF2α (500 µg) upon device removal, along with tail painting to identify estrus. In all cases, Co-Synch was not performed alongside GnRH administration. As previous studies have shown, heifers in estrus were inseminated at 60-64 hours (without GnRH), while those not in estrus received GnRH at 60-64 hours and were inseminated at 72 hours. Pregnancy diagnosis was conducted via ultrasound 30-35 days after FTAI. Pregnancy rates were similar between the J-Synch and 5-day GnRH-based protocols (57% and 55%, respectively; P=NS), but significantly lower ($P<0.05$) in heifers receiving the injectable progesterone protocol and those in the control group (44% and 44%, respectively). These differences remained consistent across heifers at 14 months and those at two years of age. Considering these results, we suggest that the 5-day GnRH-based protocol with intravaginal progesterone is a suitable treatment for FTAI in heifers without the use of estradiol. We refer to this protocol as Split-Synch 5d, as it differs from the 5-day Co-Synch in that animals are divided based on estrus manifestation on the day of FTAI, with heifers in estrus inseminated without GnRH at 60-64 hours and those not in estrus receiving GnRH at that time and inseminated 72 hours post-device removal.

In subsequent experiments, we evaluated alternatives to enhance the Split-Synch 5d protocol, aiming to improve follicular wave synchronization at protocol initiation and luteolysis at the end. Our first objective was to determine if GnRH administration at the start of the protocol was necessary. We conducted an experiment with six replicates involving 1,698 Angus heifers, with 1,205 of 13-14 months and 493 of two years old. At protocol initiation, 47% of heifers of 13-14 months and 71% of two years had a corpus luteum. All heifers received an intravaginal progesterone device and were divided into three experimental groups to receive: a single intramuscular dose of GnRH (8.4 µg) (n=565); double intramuscular dose of GnRH (16.8 µg) (n=574); or no GnRH at all (control group, n=557). Five days later, all devices were removed, and heifers received eCG (200 IU), a luteolytic dose of PGF2α (500 µg), and tail painting to identify estrus. The next morning, all heifers received a second luteolytic dose of PGF2α. Pregnancy diagnosis was conducted 30-35 days post-FTAI. Pregnancy rates were similar between heifers receiving one or two doses of GnRH at protocol initiation (63.5% and 64.6%, respectively; P=NS), but significantly higher than in the group without GnRH at initiation (52.4%; P<0.05). In conclusion, GnRH administration at protocol initiation is necessary in the Split-Synch 5d protocol, and increasing the buserelin dose does not improve pregnancy rates.

Furthermore, it remains unclear whether a single PGF2α dose is sufficient to induce luteolysis in 5-day progesterone device protocols, or if two doses are required. We designed an experiment to evaluate the need for two PGF2α doses at the end of the protocol. A total of 2,132 heifers (1,546 of 12-13 months and 586 of two years old) across seven replicates were synchronized using a Split-Synch 5d protocol. All heifers received an intravaginal progesterone device and an intramuscular GnRH injection on day 0, and a single intramuscular PGF2α dose on day 5. Pregnancy diagnosis was conducted 30-35 days post-FTAI. There was no difference in pregnancy rates between groups receiving different PGF2α doses (P=NS), regardless of the GnRH analogue used. Pregnancy rates were 49.4% in heifers treated with a single PGF2α dose, 52.5% with two doses, and 49.6% with one dose at device removal and a second dose 16 hours later (P=NS). In conclusion, a single PGF2α dose is sufficient at device removal in heifers treated with the Split-Synch 5d protocol.

Based on these experiments involving 5,777 heifers, we can conclude that: a) the Split-Synch 5d protocol appears to be the best alternative to the J-Synch protocol; b) GnRH administration at protocol initiation is necessary without the need for dose escalation; and c) a single PGF2α dose is sufficient at the end of the protocol. Figure 1 illustrates the recommended Split-Synch 5d protocol for heifers (Menchaca, 2021).

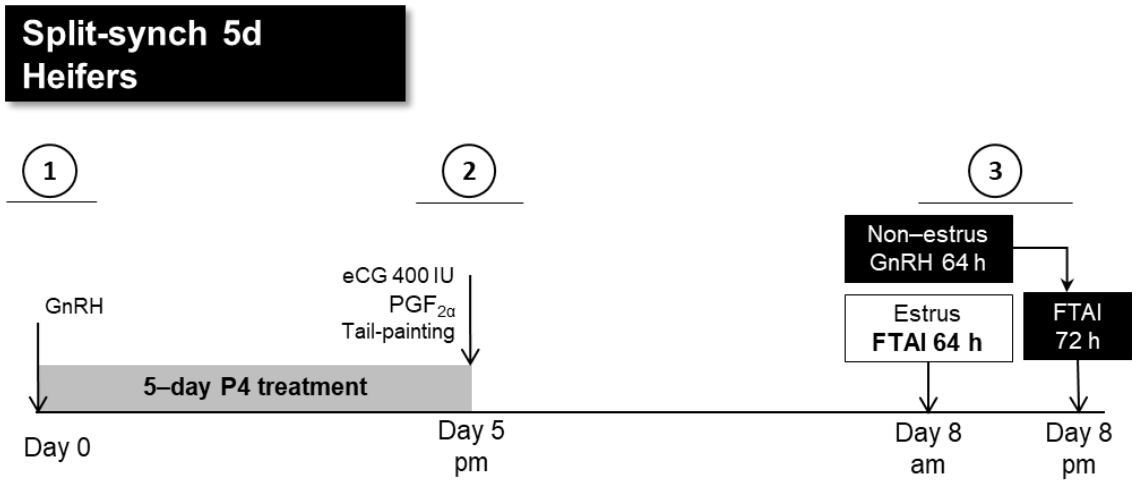


Figure 1. Schematic representation of the Split-Synch 5d protocol in heifers, as described in Menchaca (2021). Day 0: Administration of a dose of GnRH and placement of a progesterone-releasing device that remains for 5 days. Day 5 (evening): Removal of devices and administration of a luteolytic dose of PGF2α, 200 IU of eCG, and application of paint or patch at the base of the tail to identify estrus. Day 8 (early morning): Evaluation of the paint status. Heifers showing estrus are inseminated (60-64 hours after device removal), while those not showing estrus receive a dose of GnRH in the morning and are inseminated in the evening (72 hours after device removal).

Suckled cows

In those countries where estradiol salts are still available, the conventional protocol with BE and ECP continues to be the most used for synchronizing ovulation in cows with calves. This protocol requires a dose of 2 mg of BE at the beginning of the protocol (when placing the progesterone device), and a dose of 1 mg of ECP at the time of removing the intravaginal device as an ovulation inductor. Considering that this category of cows with calves represents the highest percentage of animals receiving TAI each year, the development of an alternative protocol without estradiol is of great importance.

In a first experiment conducted in cows with calves, we evaluated the use of injectable progesterone as an alternative to replace BE at the beginning of the protocol, as well as GnRH to replace the ECP administered when removing the intravaginal device. The experiment was carried out in three replicates using 1,011 Angus sucked cows that were between 60 and 90 days postpartum, with a body condition score of 4.6±0.6 (scale 1 to 8), and 18% of them having a corpus luteum at the start of the experiment. They were synchronized with a progesterone device (DIB 0.5, Zoetis) on day 0 and assigned to four experimental groups in a 2x2 factorial arrangement to receive an i.m. dose of BE (2 mg, Zoetis) or injectable progesterone (100 mg, Syntex) at the beginning of the protocol, and ECP (1 mg, Zoetis) at the time of device removal on day 8 am or GnRH (100 µg gonadorelin acetate, Zoetis) 62 h after device removal on day 7 pm. At the time of device removal, all groups received an i.m. dose of eCG (400 IU, Zoetis), PGF2α (500 µg cloprostenol, Zoetis), and paint on the tail base to identify those cows showing estrus.

FTAI was performed on day 10, cows receiving ECP were inseminated starting from 48 h after device removal, while cows without ECP were inseminated starting from 62 h. Those cows that were in heat on the morning of the day of TAI were inseminated at that time, while cows that still showed the paint (not in heat) were administered GnRH at that time and inseminated in the afternoon. The four experimental groups were composed as follows: Conventional Protocol BE+ECP (FTAI at 48-58 h after device removal, n=254); BE+GnRH protocol (FTAI at 62-72 h after device removal, n=252); P4+ECP protocol (FTAI at 48-58 h after device removal, n=247); P4+GnRH protocol (FTAI at 62-72 h after device removal, n=258), with this last group exempt from estradiol. The highest pregnancy rate was obtained in those cows that received the Conventional Protocol, BE+ECP (63%, 159/254; P<0.05) with a difference of 10 to 16% compared to the pregnancy results of the other three experimental groups (47 to 53%). When evaluating the main effect in the 2x2 factorial arrangement, it was observed that cows receiving BE had a higher pregnancy rate than those receiving injectable progesterone (58%, 293/506, vs. 49%, 247/505, respectively, P<0.05), there was no significant difference between those receiving ECP at device removal and those that did not receive it (55%, 276/501, vs. 52%, 264/510, respectively, P>0.1). In conclusion, in this experiment, an alternative protocol to the use of estradiol that is as effective as the conventional protocol with BE and ECP was not found.

Since the results with the 7 or 8-day protocols without BE at the beginning of the treatment were not satisfactory, a subsequent experiment was conducted with the aim of comparing the pregnancy rate obtained with the conventional protocol with BE+ECP against two GnRH-based protocols that prolong the proestrus and shorten the protocol duration to 5 or 6 days. A total of 914 multiparous Angus cows with calves at foot, 60 to 70 days postpartum, 16% with CL, and 3.0±0.4 body condition score (scale 1 to 8) were used. The cows were assigned to three experimental groups to receive at the time the intravaginal progesterone devices were placed (DIB 0.5, Zoetis): a) BE (2 mg, Zoetis, im), the devices were kept for 7 days (Conventional Protocol, n=308); b) GnRH (100 µg gonadorelin acetate, Zoetis), the devices were kept for 6 days (Split-Synch 6d, n=306); or c) GnRH (100 µg gonadorelin acetate, Zoetis) and the devices were kept for 5 days (Split-Synch 5d, n=300). The devices were removed in the afternoon in the Split-Synch 6d and Split-Synch 5d protocols, and in the morning of the following day in the Conventional and J-Synch protocols, so that for each replicate FTAI was performed in all cows on a single day, thus keeping the animals managed all together in a single batch. At the time of device removal, cows receiving the conventional protocol were administered an i.m. dose of ECP (1 mg, Zoetis), eCG (400 IU, Zoetis), and PGF2α (500 µg cloprostenol, Zoetis). Cows from the Split-Synch 6d and 5d protocols received 400 IU of eCG (Zoetis) at device removal, and PGF2α was administered in the Split-Synch 6d group in a single dose (500 µg cloprostenol, Zoetis) at device removal, and for the Split-Synch 5d group in two doses (500 µg cloprostenol each) with the first one at device removal and the second one 16 h later in the morning of the following day. Paint was applied to all cows when removing the device to identify those cows showing estrus. FTAI for all experimental groups was performed on the same day, early in the morning the paint status was evaluated, and those cows that had shown estrus received FTAI at that time (48 h after device removal for the Conventional Protocol, and 64 h for the Split-Synch protocols), while those that had not shown estrus yet received GnRH at that time, and

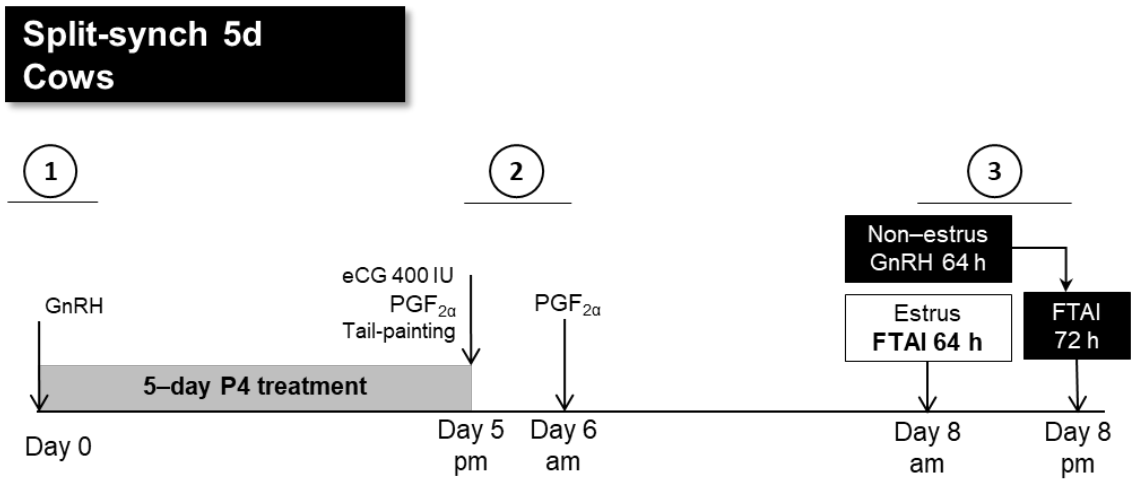
FTAI was performed in the afternoon (56 h for the Conventional Protocol and 72 h for the Split-Synch protocols). The pregnancy rate in the group that received the Conventional Protocol was similar to that in the Split-Synch 5d group (65%, 200/308 vs. 67%, 201/300; respectively; P=NS), and lower in the Split-Synch 6d group (57%, 175/306; P<0.05). In conclusion, the results indicate that the recommended estradiol-free protocol for cows with calves is the Split-Synch 5d, and that keeping the device for 6 days to apply a single dose of PGF2α decreases the pregnancy rate.

Although in heifers we have shown that with this 5-day protocol a single dose of PGF2α can be administered when removing the progesterone device, in cows it is recommended to use two doses separated by a few hours. The following experiment was conducted to evaluate the administration of PGF2α in cows with calves in a double dose at device removal or in two doses separated by 12-16 h. Angus and Hereford cows (n= 2,156) with calves at foot, 60 to 90 days postpartum, and a body condition score of 4.3±0.01, located in three establishments where used in eight replicates. In each replicate, all cows received a Split-Synch 5d protocol with a dose of GnRH (8.4 µg buserelin acetate, Pluserelin, Calier) administered on Day 0 along with the placement of a progesterone device with 0.5 g of progesterone (DIB 0.5, Zoetis). The devices were removed in the afternoon of Day 5 along with the administration of an eCG dose (400 IU, Zoetis), and the application of paint on the rump to later determine estrus manifestation. Cows were divided into two experimental groups: one group received a single administration of PGF2α with twice the usual dose (1000 µg cloprostenol, Zoetis) at device removal, while another group received this double dose separated into two injections of 500 ug of cloprostenol each (one at device removal and the other 12-16 h later on the following morning). FTAI was performed on Day 8, and early in the morning, the paint status was evaluated for both experimental groups, with cows showing estrus receiving FTAI at that time (64 h after device removal), while those that had not shown estrus yet received GnRH at that time, and FTAI was performed in the afternoon (72 h after device removal). The pregnancy rate in the group that received the double dose of PGF2α at device removal was 60.7% (651/1,072) while in the group that received the two doses separated was 69.5% (753/1,084) (P<0.05). In conclusion, in cows with calves - unlike heifers - in the 5-day protocol with GnRH, it is recommended to administer two doses of PGF2α separated by 12 to 16 hours.

Based on the experiments conducted on 4,081 cows with calves, we conclude that the best alternative to the conventional protocol with estradiol is to perform a GnRH-based protocol but maintaining a prolonged proestrus with 5 days of progesterone. In heifers, a single dose of PGF2α can be administered when removing the progesterone device, but in cows, it is necessary to administer a second dose 12 to 16 hours later. FTAI is recommended to be performed between 60 and 72 hours after device removal. This protocol is called Split-Synch 5d and is shown in Figure 2 (Menchaca, 2021).

Figure 2. Schematic representation of the Split-Synch 5d protocol for suckled cows. Day 0: Cows receive a dose of GnRH along with the insertion of an intravaginal progesterone-

releasing device. Day 5: Removal of intravaginal devices. Administration of a luteolytic dose of PGF2α, 400 IU of eCG. Application of paint or patch at the base of the tail to identify cows in heat. Day 6 (morning): Administration of a second dose of PGF2α, between 12 and 16 hours after the removal of the devices. Day 8 (early morning): Evaluation of the paint status. Insemination of cows that have shown heat at that time (60-64 hours after device removal). Day 8 (morning): Administration of a dose of GnRH to cows that have not shown heat yet. Insemination of these cows in the afternoon (72 hours after device removal).



FINAL REMARKS

In this paper we presented those FTAI protocols that do not require the use of estradiol, maintaining a simple herd management and achieving at least the same pregnancy rate as protocols with estradiol. Based on the results obtained, we can indicate that effective alternatives exist for Bos taurus cows and heifers, and we have named this protocol Split-Synch 5d. With this scheme, it is possible, like with the conventional protocol in cows and the J-Synch in heifers, to inseminate animals in heat and not in heat throughout the day, thus allowing the implementation of large-scale FTAI programs. In heifers, it is necessary to administer a dose of GnRH at the beginning of the treatment, and one dose of PGF2α at the end of the treatment is sufficient to induce luteolysis. In cows, at the end of the protocol, it is recommended to administer two doses of PGF2α with a 12 to 16-hour interval, with the first dose at the time of device removal. This series of experiments conducted under management conditions where a good portion of cows and heifers are in anestrus demonstrate the efficacy of this estradiol-free protocol for beef cows.

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Reproductive management strategies to place high-producing dairy cows in the high fertility cycle.

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Abstract: Reproductive efficiency is related with the economics of dairy herds, and in this aspect it is important to achieve high 21-d pregnancy rates, reducing calving interval and days in milk of the herd, improving profitability. To achieve high 21-d pregnancy rates, the service rate and pregnancy per artificial insemination (P/AI) must be increased. Currently, there are adjustments in timed-artificial insemination (TAI) protocols and use of presynchronization strategies that are able to increase P/AI, and implementation of a systematized reproductive program that combines the use of TAI with efficient detection of estrus can result in high reproductive indexes associated with high milk production, having the majority of the cows in the "High Fertility Cycle", which is the concept of the cows achieving subsequent short calving intervals.

Keywords: cattle, fertility, timed-AI, dairy cows, management, reproductive tools

Introduction

Genetic selection for milk production has been associated with poorer fertility or lower reproductive performance, depending on how cows are handled for comfort, nutrition, health status and reproduction. However, with proper management aiming to optimize environment and comfort conditions, associated with well-conducted reproductive programs that guarantee the likelihood for cows to become pregnant early in lactation, high milk-producing herds can achieve high reproductive performances. In order to obtain satisfactory reproductive indexes, a holistic vision within a herd is necessary, considering key factors that impact reproduction, besides reproductive management.

The overall objective of reproductive managements is to generate high 21-d pregnancy rates (21d-PR) and an ideal calving interval (CI) depending on the herd. The 21d-PR is calculated based on service rate (SR) and pregnancy per artificial insemination (P/AI), in which the herds must aim to maximize the efficiency in inseminating eligible cows, and to increase the likelihood of cows to become pregnant among inseminated cows.

Timed-AI (TAI) protocols have been increasingly used in reproductive management of dairy cattle (Wiltbank and Pursley, 2014). At the beginning, the impact of TAI was especially due to increase in SR, because in the early days, the fertility of traditional Ovsynch programs was similar as when cows were inseminated after detection of estrus (Pursley et al., 1997; Rabiee et al., 2005). A few years after the development of Ovsynch, TAI protocols known as estradiol (E2) and progesterone (P4)-based protocols were created (Barros et al., 2000; Bó et al., 2000; Bó et al., 2003 Baruselli et al., 2004).

In the last two decades, the TAI programs were extensively studied by many research groups around the world, and adjustments have been established to improve P/AI. Moreover, fertility programs were created, which include a presynchronization

prior to the breeding protocols initiated with gonadotropin releasing-hormone (GnRH), and improve the hormonal milieu during development of the synchronized preovulatory follicle and increase P/AI (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008; Santos et al., 2017). Besides the use of these fertility programs for first postpartum AI, "aggressive" reproductive management strategies for reinseminations should be employed aiming to maximize the number of cows pregnant early in lactation.

Besides reproductive programs, there are numerous other factors that impact fertility, such as how the transition period is managed. The success in terms of health issues (Santos et al., 2010; Carvalho et al., 2019), energy and body condition score (BCS) balance (Carvalho et al., 2014; Barletta et al., 2017) reflects the likelihood of cows to become pregnant early in lactation. Based on recent data, the impact of BCS change and health issues during the transition period on fertility are extremely relevant, and the concept of the "High Fertility Cycle" is well discussed by Middleton et al. (2019).

Importance of achieving high reproductive performance

The decision of which reproductive management will be used in a specific herd depends on several factors such as intensity of TAI use, SR, P/AI at estrus or at TAI, compliance of procedures, and strategies for estrus detection (Giordano et al., 2011, 2012a; Kalantari and Cabrera, 2012; Ricci et al., 2020). However, an essential concept is that greater 21d-PR is associated with reduced days open, CI, and average days in milk (DIM) of the herd (Giordano et al., 2012a), which is economically positive, since cows in the first third of lactation provide greater income over feed cost compared to cows in the middle or at the end of lactation (Ribeiro et al., 2012).

Thus, one of the main goals of the reproductive management in a dairy herd is to maximize the number of cows pregnant early in lactation. The reduction in days open is associated with a reduction in CI, which promptly diminishes the number of days in the dry period, when the cows are not producing milk in the herd. A smaller CI is associated with greater milk production per day and in whole lactation. For instance, Ribeiro et al. (2012) reported that a reduction in CI of ~60 d increased milk production per day (1.51 and 1.11 kg/d) and in whole lactation (~498 and ~366 kg/lactation) in both high-producing (12,500 kg in 305 d of lactation) or moderate-producing herds (9,000 kg in 305 d of lactation), respectively.

Another important benefit of high reproductive efficiency is related to the culling rate and profile of dairy herds. In 2018, out of 1,035,647 culls reported in US herds, reproductive problems represented 15.3% (DHIA, 2018). The greater the 21d-PR, the sooner the cows become pregnant and the greater is the proportion of cows pregnant within the herd. Therefore, the reproductive
related culling is decreased, and more importantly, the voluntary culling related to lower milk production, udder issues, genetics, foot and leg or other problems, is increased. This happens because more cows are pregnant within the herd and the number of heifers/primiparous cows entering in reproduction and lactation increases.

What is important to occur during the TAI programs to optimize fertility?

Protocols for TAI can be divided into two pharmacological bases: 1) The Ovsynch-type protocols, and 2) E2 plus P4-based protocols. Regardless of the hormonal combinations used in a particular protocol, the physiological objectives of them are to

synchronize emergence of a new follicular wave, to control circulating P4 concentrations during the time of development of the preovulatory follicle and near the time of AI, to efficiently regress a CL in an adequate timing, to induce a synchronized ovulation of a follicle with adequate size and age, and producing adequate amount of E2 at the end of the protocol.

A prolonged period of dominance can reduce fertility due to a negative effect on oocyte quality and viability (Revah and Butler, 1996). For example, Cerri et al. (2009) reported approximately 80% of grade 1 and 2 embryos (viable) in cows with 5.5 to 6.0 d of follicle dominance. On the other hand, only 45% of embryos were classified as viable in cows with prolonged follicle dominance (8.5 to 11.5 d). It is important to mention that fertilization was not affected by follicle age (Cerri et al., 2009). In a study that carried out ovarian dynamics during an E2/P4 TAI protocol, cows without follicle wave emergence at the beginning (ovulating persistent follicles at the end) had lower P/AI compared to cows in which follicle wave emergence was synchronized (21 vs. 43%; Monteiro et al., 2015). In contrast, Vasconcelos et al. (2001), in the attempt to characterize the effect of the follicular dominance period on fertility, aspirated follicles from dairy cows on days 3 to 4 after the first GnRH of the Ovsynch in order to produce a younger and smaller ovulatory follicle. In fact, follicles from cows submitted to aspiration were smaller than from the control cows (11.5 vs. 14.5 mm). However, the fertility of cows that ovulated younger follicles was much lower when compared to cows submitted to Ovsynch without aspiration on day 3-4 (14.3% vs. 47.6%). The authors reported that this lower fertility may have occurred due to lower circulating concentrations of E2 during the pre-ovulatory period of these cows, and due to lower circulating concentration of P4 after AI, related to a smaller CL. Other authors have correlated a larger ovulatory follicle size to greater circulating E2 previous to AI and to a larger CL size and circulating P4 after AI (Sartori et al., 2002; Bello et al., 2006). In order to establish a correlation between ovulatory follicle size and fertility, Souza et al. (2007) evaluated the effect of ovulatory follicle size on P/AI, which was greater in cows that ovulated follicles of intermediary size (15-19 mm, 47%) compared to those ovulating smaller (< 14 mm, 36%) or larger (> 20 mm, 38%) follicles. Therefore, the strategies used to initiate TAI programs must be efficient in promoting a new follicular wave emergence at the beginning, in order to ovulate at the end a follicle of an ideal age/size (not too old, not too young).

A well-known factor that influences fertility in high producing dairy cows is circulating P4 concentration during development of the preovulatory follicle. Lower circulating P4 during follicular growth, either due to an anovulatory condition (Chebel et al., 2010) or due to the higher catabolism of this hormone in high-producing cows (Sangsritavong et al., 2002; Sartori et al., 2002) is associated with greater pulsatility of LH, which can result in premature resumption of oocyte meiosis and germinal vesicle breakdown, decreasing oocyte quality, and consequently fertility (Revah and Butler, 1996). A study (Rivera et al., 2011) reported that cows with milk production over 40 kg/d that were superovulated during the first follicular wave had a greater percentage of degenerate embryos (23.5%) compared to cows superovulated during the first follicular wave but with P4 supplementation (7.1%) or those superovulated during the second follicular wave (3.9%). Moreover, the percentage of transferable embryos was much greater after superovulation during the second follicular wave (88.5%) and the first wave with supplementary P4 (78.6%) compared to superovulation during the first

follicular wave (55.9%). An elegant study evaluated the effect of circulating P4 concentration on embryo quality of cows synchronized and with single ovulation (Wiltbank et al., 2011), in which the ovulatory follicle developed under a higher or lower circulating P4 milieu. Although fertilization was similar (78% on average), percentage of grade 1 and 2 embryos (high quality embryos) was greater for cows ovulating follicles that developed under higher P4 (86.5%) than follicles that developed under lower P4 (61.5%). Moreover, cows with higher circulating P4 had fewer degenerate embryos (8.1%) than cows with lower circulating P4 (34.6%). Many studies have reported greater P/AI when cows were submitted to TAI programs in which a CL was present and/or the P4 milieu during follicle development was high (Bisinotto et al., 2013, 2015a; Melo et al., 2016). In a compilation of data from studies of our lab, presence of CL at the beginning of TAI protocols and/or at the time of PGF increased P/AI in 15-24%, and the best fertility was achieved when CL was present at both times of the protocol.

Another important aspect of circulating P4 concentration during TAI programs is related with double/multiple ovulation and twinning. Double ovulation is more frequent when there is low circulating P4 during the protocol (Martins et al., 2018; Carvalho et al., 2019) and in cows with higher milk yield (Fricke and Wiltbank et al., 1999; Lopez et al., 2005). Another factor that influences double ovulation is parity, in which multiple ovulations have been described to be more frequent in multiparous compared to primiparous cows (Macmillan et al., 2018), and this can be explained by the greater milk production in multiparous cows. Double ovulation in dairy cattle is undesirable because it increases the incidence of twin pregnancies (Fricke et al., 2001), which are associated with calving problems, calf mortality, freemartin, and problems with calf development. Moreover, twinning is associated with greater pregnancy loss after 30 d of pregnancy (López-Gatius et al., 2002; Silva del Río et al., 2009). Thus, hormonal strategies in TAI programs must assure adequate circulating P4 during the development of the preovulatory follicle, aiming to increase P/AI and reduce pregnancy loss.

Many studies presented results on the relationship between circulating P4 concentrations near TAI and ovulation or fertility (Souza et al, 2007; Brusveen et al., 2009; Lopes et al., 2013; Carvalho et al., 2015; Monteiro et al., 2015; Colazo et al., 2017). All of them reported a dramatic negative impact on fertility of residual P4 near the time of the second GnRH of the Ovsynch or at the time of AI. For example, in a large data set compiled by Carvalho et al. (2018), there was a 66% relative decrease in P/AI for cows with P4 ≥ 0.4 ng/mL (14%; 161/435) than for cows with P4 < 0.4 ng/mL (41%; 1,125/2,713) at the time of the second GnRH treatment (G2) during the Ovsynch protocol. This residual P4 near AI is due to a lack of complete luteolysis after the PGF treatment during the protocols, which may occur in 13 to 44% of cows (Heidari et al., 2017; Barletta et al., 2018), and is more problematic when young CL are present at the time of PGF, due to their lower responsiveness to a single treatment with PGF (Nascimento et al., 2014). Therefore, new strategies have been used in TAI programs to overcome the issue of incomplete CL regression at the end of the protocol.

Programs for TAI were developed to avoid the need to detect estrus prior to AI, but expression of estrus at the end of TAI protocols are associated with greater P/AI and lower PL. In general, cows that express estrus by the time of TAI achieve greater P/AI, in both Ovsynch type (Bisinotto et al., 2015b) or E2/P4-based protocols (Pereira et al.,

2016). Another positive effect of estrus expression is the decrease in PL, as reported for example, by a study with 5,430 cows, in which cows expressing estrus had ~28% lower PL (Pereira et al., 2016). Expression of estrus can increase fertility due to greater ovulation incidence even when TAI protocols are used (Galvão et al., 2004). Moreover, estrus is related with circulating E2, which is greater when cows have bigger follicles at the end of the protocols (Souza et al., 2007), and higher circulating E2 before AI is also associated with greater fertility (Bello et al., 2006). Adequate circulating E2 prior to AI is associated with a differentiated expression of genes in the endometrium and conceptus, favorable to pregnancy (Davoodi et al., 2016), and to adequate gamete activity and transport (Cascieri et al., 1976). Thus, one aspect that should be considered in TAI programs is the expression of estrus at the end of the protocols, and this is related with the strategy that is used to induce final ovulation.

There are two main strategies used to initiate TAI protocols and to synchronize a new follicular wave emergence. The first one aims to synchronize emergence of a new wave by causing atresia of the follicles present in the ovaries due to a negative feedback in follicle stimulating-hormone (FSH) and luteinizing hormone (LH), promoted by a combination of the increase in circulating E2 (from an E2 ester) and P4 (from intravaginal P4 implants, IVP). This is the base of the initiation of E2-P4-based protocols. The second strategy, which is the base of Ovsynch-type protocols, promotes a new follicular wave emergence after an ovulation of a dominant follicle responsive to an exogenous GnRH treatment.

The most used E2 ester along with P4 implants on d0 of TAI protocols is estradiol benzoate (EB) with the dose of 2 mg. However, this strategy did not properly synchronize emergence of a new follicular wave in more than 25% of lactating dairy cows (Monteiro et al., 2015). In fact, Melo et al. (2018) reported 35% of cows ovulating persistent follicles at the end of a protocol starting with EB and a P4 implant. So, this issue can impair fertility considering that older/persistent follicles may ovulate overstimulated oocytes and, therefore, result in poorer embryo development in lactating dairy cows. Another potential problem with E2/P4-based protocols is that treatment with EB at the beginning is related with greater incidence of CL regression between d0 and the time of PGF treatment, decreasing the percentage of cows with CL and number of CL at PGF, which is related to lower circulating P4 during development of the preovulatory follicle (Melo et al., 2016; Consentini et al., 2018, 2020), compromising fertility. About ~40% of the cows that had a CL present on d0 underwent CL regression between d0 and PGF when received EB at the beginning (Monteiro et al., 2015; Melo et al., 2016; Consentini et al., 2020).

When the TAI protocol starts with GnRH, the objective is to induce ovulation, that results in emergence of a new follicular wave and a new a CL during the protocol, increasing circulating P4 during follicle growth. In fact, ovulation after d0 increased circulating P4 at PGF in many studies (Carvalho et al., 2015; Giordano et al., 2013; Melo et al., 2018), as well as P/AI (Giordano et al., 2013). Ovulation after d0 primarily impacts P/AI in cows initiating the protocol without CL or with low circulating P4 (Giordano et al., 2013; Consentini et al., 2020). Since ovulation after d0 is associated with greater circulating P4 during follicle development and greater P/AI, TAI programs must aim to maximize this response. One strategy is the use of presynchronization. The other approach to increase ovulation after d0 of a TAI protocol is related to the dose and analogue of GnRH. When Giordano et al. (2012b) increased the dose of gonadorelin

acetate from 100 to 200 µg, there was a greater LH peak, and that strategy was more pronounced when cows had higher circulating P4, due to an inhibitory effect of P4 on the GnRH-induced LH peak. In fact, in a study using nonlactating Holstein cows, the dose of 100 µg of Gonadorelin promoted 58.1% of ovulation in cows with a 7-d old CL present compared to 95.5% of ovulation in cows without CL (Silva et al., 2023). When comparing two analogues of GnRH, studies from our laboratory (Silva et al., 2024) have shown that 100 µg gonadorelin acetate produced a lower LH peak compared to 10 µg buserelin acetate in Nelore (Bos indicus) heifers (5.4 vs. 11.7 ng/mL) and cows (3.4 vs. 6.9 ng/mL) on day 7 of the estrous cycle. When the dose of those two analogues was doubled, buserelin increased the LH peak in heifers (11.7 vs. 23.2 ng/mL) and cows (6.9 vs. 13.2 ng/mL), whereas the double dose of gonadorelin only increased the LH peak in cows (3.4 vs. 6.3 ng/mL) but not in heifers (5.4 vs. 5.2 ng/mL). Considering the main effects of the study, buserelin induced a greater LH peak and ovulation compared to gonadorelin (Silva et al., 2024). Other studies have reported greater efficiency of buserelin and lecirelin compared to gonadorelin (Chenault et al., 1990; Picard-Hagen et al., 2015). Therefore, it is recommended initiating TAI protocols with GnRH instead of EB. Moreover, it should be considered doubling the dose of GnRH in order to maximize the ovulatory response at the beginning of a TAI protocol, especially in cows expected to have CL on d0.

When considering the use of IVP, is that P4 implants do not induce cows to have circulating P4 as high as when they have an active CL. For example, in the study by Sartori et al. (2004), circulating P4 on d7 and d14 of an estrous cycle in lactating dairy cows was 2.1 and 4.2 ng/mL, respectively. In contrast, in a study from our laboratory, when comparing two commercial P4 devices (1.9 and 2.0 g of P4) in postpartum cows without CL and producing 40 kg of milk per d, there was a peak of circulating P4 similar between devices (1.6 ng/mL) and the mean P4 during 9 d of insertion was 0.85 ng/mL (unpublished results). Bisinotto et al. (2013, 2015a) reported greater circulating P4 in cows with CL during the protocol compared to those without CL but supplemented with one or two P4 devices (1.38 g), despite the fact that P4 supplementation increased circulating P4 to 1.5-1.9 ng/mL. Therefore, it is important to guarantee that a high proportion of cows initiate the TAI protocols with CL, either by decreasing anovulatory conditions or by using presynchronization programs. A study (Bisinotto et al., 2015a), with more than 600 cows per group, compared cows initiating Ovsynch with CL to cows without CL, supplemented or not with two P4 implants with 1.38 g of P4, each. Cows without CL at the beginning of the protocol had the lowest fertility (31.3%), but P/AI on d32 did not differ between cows with CL and those without CL and P4 supplementation (38.4 and 42.2%, respectively). In a study with ~160 cows per group, using E2/P4-based TAI protocols, two P4 implants during the protocol tended to increase P/AI on d60 of ovulated cows compared to one implant (48.1 vs. 37.7%; Pereira et al., 2017).

It has already been discussed the importance of a complete luteolysis at the end of a TAI protocol, to optimize fertility. In order to achieve this goal, two strategies have been used: 1) Increasing the dose of PGF (Giordano et al., 2013; Barletta et al., 2018), and 2) Adding a second treatment of PGF, in general, 24 h after the first one (Brusveen et al., 2009; Carvalho et al., 2015; Wiltbank et al., 2015; Santos et al., 2016; Heidari et al., 2017; Barletta et al., 2018). Increasing the dose of cloprostenol sodium from 500 to 750 µg during a Double-Ovsynch program increased the percentage of multiparous

cows with complete luteolysis (87.7 vs. 79.2%) but not primiparous cows (92.8 vs. 89.7%) in a study from Giordano et al. (2013). Interestingly, doubling the dose of dinoprost tromethamine from 25 to 50 mg during the Ovsynch protocol (Barletta et al., 2018) did not increase the percentage of cows with complete luteolysis (88 vs. 88%) and did not increase P/AI (30.2 vs. 32.4%). However, two treatments with PGF 24 h apart increased the proportion of cows with complete luteolysis (88 vs. 94%) and increased P/AI (30.7 vs. 35.4%).

Synchronized ovulation of the dominant follicle at the end of TAI protocols is a particular aspect that can influence fertility in TAI programs, and it can be induced with E2 esters, such as EB or E2 cypionate (EC; Melo et al., 2016), or with GnRH, as in Ovsynch (Pursley et al., 1995). The use of EC is convenient because it can be administered concomitant with the final PGF of the protocol and/or P4 implant withdrawal (Melo et al., 2016). However, the timing of ovulation induced by EC is more variable than when GnRH is used (Pancarci et al., 2002; Souza et al., 2009). On the other hand, when GnRH is used at the end of TAI protocols, in order to achieve the best fertility outcomes, cows need to be handled one more time and expression of estrus is reduced, which could compromise fertility. Although when using EC 48 h before TAI as ovulation inducer results in more cows in estrus prior to AI compared to GnRH given 16 h prior to AI, these traditional strategies, in general, provide good and similar overall fertility and can be chosen by dairy operations according to management and costs.

How to improve performance of high producing dairy herds using reproductive programs?

It is important for dairy operations to implement reproductive managements that increase the likelihood of cows to become pregnant early, as well as use TAI programs that increase fertility. With this in mind, an interesting strategy is to inseminate cows for first service using TAI, especially because service rate is increased due to insemination of cows that would not be detected in estrus or anovular cows. Therefore, almost all cows have the first opportunity to conceive right after the end of the voluntary waiting period (VWP). In addition, fertility programs developed in the past years, mostly used for first TAI, that include presynchronization prior to the breeding protocols promote superior fertility in comparison with strategies that inseminate cow by estrus (Santos et al., 2017; Carvalho et al., 2018).

When cows do not become pregnant at first service postpartum, they must be rapidly identified as nonpregnant and re-inseminated. Therefore, to shorten the interbreeding interval, herds can detect estrus for second and greater services, although the success of this strategy rely on the service rate and the efficiency of the herd in detecting estrus. Alternatively, insemination of nonpregnant cows can be performed after resynchronization of ovulation using TAI protocols (commonly termed Resynch), usually initiated at the time of or before the nonpregnancy diagnosis (NPD; Lopes et al., 2013; Giordano et al., 2016; Wijma et al., 2017).

The use of estrus detection strategy and/or TAI depends on many factors such as efficiency of the herd to detect estrus and achieve high service rates, use of activity monitors or other tools such as tail paint or patches, milk production, cyclicity of the cows, compliance on estrus detection or TAI programs, and the intrinsic criteria of the owners to choose the reproductive management strategies.

Reproductive programs for first postpartum AI can be based on the use of a presynchronization strategy in order to ensure that most of the cows initiate the breeding protocol (initiated with GnRH) at an ideal stage of the estrous cycle (6-8 d), in which cows have a ~7 d old CL and a follicle responsive to the first GnRH, improving the P4 milieu during development of the preovulatory follicle, overall synchronization, and fertility. One of these programs is the G6G or G7G. Commonly, cows receive a PGF treatment and 2 d later a GnRH treatment, 6 or 7 d before initiating the Ovsynch protocol. Therefore, the G6G/G7G should be efficient in having more cows in the ideal stage of the estrous cycle to initiate the Ovsynch since ovulation to the presynchronization is induced and controlled by GnRH treatment. In addition, the inclusion of GnRH during the presynchronization may benefit anovular cows. The G6G/G7G is commonly used in commercial dairy herds and several studies have tested this strategy (Peters and Pursley, 2002; Yousuf et al., 2016; Heidari et al., 2017). Bello et al. (2006) reported greater ovulation to the first GnRH (85 vs. 54%), response to PGF (96 vs. 69%), synchronization rate (92 vs. 69%), and greater P/AI (50 vs. 27%) in cows submitted to G6G compared to Ovsynch initiated at random days of the estrous cycle. Another fertility program is the Double-Ovsynch (DO, Souza et al., 2008). The DO was nicely developed thinking in optimizing the response to Ovsynch hormonal treatments, overall synchronization, and hormonal milieu during follicle development. In the original study from Souza et al. (2008), when compared to Presynch-Ovsynch (PO; Moreira et al., 2001), DO decreased percentage of cows with P4 < 1.0 ng/mL (9.4 vs. 33.3%) at the time of the first GnRH of the breeding Ovsynch, increased circulating P4 at PGF (4.2 vs. 3.2 ng/mL), and increased P/AI (49.7 vs. 41.7%). In a study aiming to compare DO to PO in relation to circulating P4 concentrations and ovulation to GnRH treatments (Ayres et al., 2013), 94% of the cows in DO had CL at the time of the first GnRH compared to 68% in PO program. Moreover, ovulation to the first GnRH was greater in DO (80%) compared to PO (69.9%), and percentage of cows with P4 ≥ 1.0 ng/mL at PGF was greater in DO than PO (88 vs. 76%).

Recent experiments from our laboratory have used a presynchronization strategy prior to breeding protocols that are initiated with GnRH (Consentini et al., 2019). The presynchronization was based on E2 and P4, initiating with a P4 implant that was removed 7 d later, concomitant with treatments with PGF and EC to induce estrus and ovulation. Eight to 10 d later, the cows were treated with GnRH to initiate the first postpartum TAI protocol and an intravaginal P4 implant that was kept for 7 or 8 d. One d before and at the time of P4 implant removal, PGF treatments were given. Ovulation at the end of the protocol was synchronized with EC (given at the time of P4 implant withdrawal), GnRH (given 16 h prior to TAI), or both. The P/AI varied from 32 to 58% among six farms, with an overall P/AI of 43%. Compared to regular TAI protocols that were initiated at random stages of the estrous cycle, the fertility program significantly increased P/AI (unpublished data).

Therefore, in order to improve first service postpartum aiming to maximize SR and increasing the likelihood of cows to become pregnant early in postpartum, it seems to be mandatory, in most of the cases to use TAI. Moreover, the use of the so-called fertility programs increases the odds of cows to conceive at first postpartum AI, due to greater response to hormonal treatments of the breeding protocols and overall synchronization, and better hormonal environment during follicle development.

Figure 1 represents fertility programs that have been discussed in this manuscript.

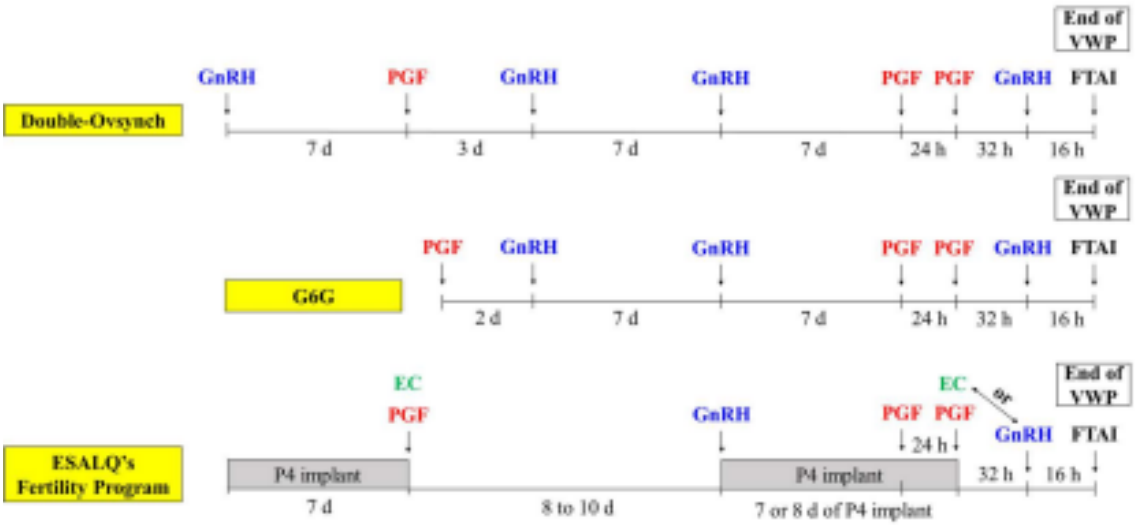

Figure 1. Hormonal treatments of fertility programs that can be used for first fixed-time artificial insemination (TAI) postpartum in lactating dairy cows.
Abbreviations: voluntary waiting period (VWP), gonadotropin-releasing hormone (GnRH), prostaglandin F2α (PGF), progesterone (P4), and estradiol cypionate (EC).

After submitting cows to the first postpartum AI, it is imperative to identify nonpregnant cows as soon as possible and to reinseminate them as early as possible. The most common strategies to reinseminate nonpregnant cows in dairy herds are either detecting estrus or inseminating them at fixed-time after NPD. Several strategies were developed to increase reinsemination rates of cows by detection of estrus (Chebel et al., 2013; Bruno et al., 2014; Giordano et al., 2015). In general, it was concluded that, in herds with good estrus detection rates and good fertility at AI by estrus, the reproductive performance can be similar to those using or including TAI. However, the herd will always have less control of the interval between inseminations, which can be longer than when submitting cows to TAI resynch programs.

Regarding resynchronization of ovulation to reinseminate cows using TAI, many studies were carried out in the past to either understand the physiology or to improve the efficiency of resynch programs. It is common to initiate the resynch protocol at the time of the NPD. However, there are strategies that initiate the TAI protocol before NPD, which include presynchronizations and/or use P4 supplementation (Fricke et al., 2003; Bisinotto et al., 2010; Giordano et al., 2012c; Bilby et al., 2013; Lopes et al., 2013; Carvalho et al., 2015; Wijma et al., 2017; Wijma et al., 2018; Sauls-Hiesterman et al., 2020).

Final considerations

This manuscript aimed to highlight important aspects related to reproductive efficiency in modern dairy herds, with the aim of maximizing number of cows pregnant early in lactation. Before considering reproductive management strategies, it is particularly important to have a holistic vision of the factors that affect reproductive

performance, in which the importance of the transition period due to its impacts on productive and reproductive performances of high-producing dairy cows should be focused, as well as the likelihood of cows to be in the "High Fertility Cycle". This manuscript discussed key aspects that are critical during TAI programs aiming to optimize fertility, as well as hormonal manipulations, emphasizing the comparison of hormonal treatments and the fine adjustments that are performed during TAI programs to optimize reproductive efficiency and profitability. Lastly, specific aspects of reproductive management for first service were discussed, highlighting the use of fertility programs that maximize SR as well as P/AI. Moreover, reinsemination strategies were presented, considering detection of estrus and TAI programs for cows that did not conceive to the previous AI. Hopefully, data presented and discussed in this manuscript will contribute to improvements in productive and reproductive efficiency of dairy operations as well as to stimulate new researches in this exciting area of science.

Acknowledgements: The authors were supported by scholarships from the National Council for Scientific and Technological Development (CNPq, Brasília, Brazil), the Coordination for the Improvement of Higher Education Personnel (CAPES, Brasília, Brazil), or from the São Paulo Research Foundation (FAPESP, São Paulo, Brazil). Some of the data presented in this manuscript were from studies supported by a grant from FAPESP (2018/03798-7).

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Sheep and goats

Sustainable control of caprine reproduction through sociosexual interactions

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Background

Reproductive seasonality is a characteristic of male and female goat breeds from temperate and subtropical latitudes. The sexual season, charactrerized by maximal spermatogenetic and sexual behavior activities in males and in estrus behavior and ovulatory cyclicity in females, generally occurs in autumn and winter. However, males are generally starting and ending earlier their sexual season than females, this advance being more marked in subtropical latitudes (i.e. 3 *vs* 1 month). This reproductive seasonality causes a seasonal production of milk and meat. Regardless of the latitude of origin of the goats, this seasonality is, in both sexes, mainly synchronized by the annual variations in photoperiod, which induce changes in estradiol and testosterone negative feedbacks on LH secretion, constituting the main neuroendocrine mechanism responsible for reproductive seasonality. Nonethless, the sociosexual interactions between bucks and goats and between bucks themselves can break the natural inhibition of sexual activity during the seasonal sexual rest. Indeed, the introduction of a male into a group of seasonal anestrous goats immediately leads to a resumption of the activity of the hypothalamic-pituitary-gonadal axis, allowing females to display estrus and ovulations within few days after joining (Walkden-Brown et al., 1999). This is the "short-term male effect". Recently, we described that the permanent presence of bucks made sexually active by photoperiodic treatments stimulate sexual activity during seasonal anestrus and allows females to ovulate throughout the year (Delgadillo et al., 2015). This is what we called the "long-term male effect". Finally, we recently described that, as in females, the introduction of a sexually active buck into a group of seasonally inactive bucks in sexual rest immediately leads to a resumption of the activity of the hypothalamic-pituitary-gonadal axis, improving spermatogenetic and sexual behavior activities of recipient bucks. This is what we called the “buck-to-buck-effect”, or more generally, the “male-to-male effect” (Delgadillo et al., 2022). These three sexual biostimulation techniques to control caprine reproduction in a sustainable way, without the use of exogenous hormones, will be described in the present article.

The short-term male effect

The introduction of a male into a group of seasonal anestrous goats stimulates immediately the secretion of LH, then followed by ovulation generally associated with estrous behavior, within the first five days after joining. The first ovulation occurs 2-5 days after joining, but some ovulations are not associated with estrous behavior. Regardless of the presence or absence of estrus, most females have a short ovulatory cycle and ovulate again 6-9 days later; this second ovulation is generally associated with estrous behavior and followed by a normal luteal phase duration. Most females,

therefore, can become pregnant at this second male-induced ovulation following the short cycle. This is the "short-term male effect", which has been widely studied by the scientific community and used to induce and synchronise estrus and ovulations in anestrous goats since the 60's. A limit of this "short-term male effect" is that when used in strongly seasonal breeds, the sexual response of females is dramatically reduced, or even null, when teasing is performed during the mid-anestrus (Restall, 1992). This response is improved when using bucks made sexually active during the sexual rest by previous exposure to two months of artificial long days in autumn and winter followed by natural photoperiod. This photoperiodic treatment stimulates their LH and testosterone secretion, improves their spermatogenetic activity, sexual behavior and sexual odor about 6 weeks after the end of the long day period, rendered them much more efficient to trigger the endocrine and sexual activites in seasonally anestrous goats than untreated, sexually inactive bucks. Indeed, in March, April and May, the proportions of goats that displayed estrous behavior or ovulations (>80 %) and kidded (> 70%) were much higher in females joined with sexually active bucks than in those joined with sexually inactive bucks (<20%; Delgadillo, 2011). In June, most goats respond to the introduction of untreated bucks who display intense sexual activity because the sexual season of these males has already begun (Figure 1). In addition, the sexually active bucks advance billy goats puberty and reduce the duration of postpartum anestrus (Delgadillo et al., 2020). Interestingly, the sexually active bucks have allowed to reject some "dogmas" about the "short-term male effect". One of these dogmas indicated a prior separation of the two sexes before achieving the male effect to successfully induce estrus and ovulations. However, the proportions of females that ovulated when joined with the sexually active males did not differ between females separated (40 days) or not from the bucks (>85 %; Zarazaga et al., 2017). Therefore, when sexually active bucks are used, a prior separation between sexes is not necessary. These results are important from a practical application of the "short-term male effect" because it facilitates its use by not having to separate the sexes. Altogether, these findings indicate that sexually active bucks are especially efficient to stimulate the endocrine and reproductive activities of goats during the seasonal anestrus and indicate that the intensity of males' sexual behavior is a crucial factor that should be taken into consideration to successfully achieve the "short-term male effect".

Considering that the estradiol negative feedback on LH secretion is the main neuroendocrine mechanism responsible for seasonality, it is likely that the sexually active bucks are able to reduce or overcome this negative feedback. Indeed, in ovariectomized goats bearing subcutaneous implants releasing estradiol (OVX+E), exposure to sexually active bucks increased LH secretion within 15 min of exposure, but exposure to the sexually inactive males did not stimulate this secretion. Therefore, the sexually active bucks probably modify the estradiol negative beedback on LH secretion and activated the kisspeptin neurons located in the arcuate nucleus, which control GnRH secretion (Delgadillo et al., 2020).

The long-term male effect

Recently described, although it undoubtedly sometimes occurs in natural conditions, the "long-term male effect" probably represents one of the regulators of the reproductive seasonality in goats.

Permanent presence of males among females

The permanent presence of bucks among a group of females reduce the duration of anestrus because the sexual season begins earlier and end later than in goats isolated from males throughout the year. However, it does not prevent the appearance of the seasonal anovulation (Restall, 1992). These findings were obtained when bucks were subjected to the natural variations of photoperiod, exhibiting periods of sexual rest, which roughly coincide with those of females. This latters indicates a possible influence of males on the triggering of the seasonal ovulatory activity of females when the two sexes are in permanent contact.

Onset of ovulatory activity during season anestrus

Considering that about 6 weeks are necessary after the end of long days for the sexual activity of males to begin, and that sexual behavior is a crucial factor to successfully achieve the "short-term male effect", we wonder what would happen if goats were in contact with photoperiodic-treated bucks when their sexual activity starts after the long days? Would these females ovulate? During these 6 weeks after the end of the long days, the bucks are still in seasonal sexual rest with very low sexual activity and if they are introduced into a group of females at the beginning of anestrus, for example in February, they do not cause a "short-term male effect" (and also because goats are still cycling). But their gradual resumption of sexual activity in March, induced by the previous photoperiodic treatment, gradually leads to the ovulatory activity of the females and all of them ovulated in April, in the middle of the anestrus period (Delgadillo et al., 2015). This is what we called the "long-term male effect".

Suppression of seasonal anovulation

Considering the previous results, we wonder what would happen if goats remained in permanent contact, during seasonal anestrus, with sexually active males? Would these females maintain ovulatory activity throughout the year? From January and for a period of 18 months, a group of goats remained isolated from bucks, while another one remained in permanent contact with vasectomized control bucks submitted to the natural photoperiod, and displayed periods of sexual activity and sexual rest. Another group of goats remained in permanent contact with vasectomized bucks made sexually active during the period of sexual rest (January-June) by previous photoperiodic treatments applied during autumn and winter (three groups of bucks were used successively). As expected, a longer duration of anestrus (March-September) was observed in goats isolated from bucks, than in those in permanent contact with the control bucks (April-June). By contrast, almost all (12/14) of the goats that remained in permanent contact with sexually active bucks were in cyclical ovulatory activity for the entire duration of the experiment, including the two seasons of anestrus observed in isolated females. To demonstrate that it was indeed the presence of the bucks which induced permanent sexual activity, they were removed from half of the cyclic females in May of the second year; the activity then stopped immediately (Figure 2; Delgadillo et al., 2015).

This "long-term male effect" using sexually active bucks, as in the case of the "short-term male effect", passes through the stimulation of the central nervous system.

Indeed, in OVX+E goats kept in permanent contact with the sexually active bucks, the plasma concentrations of LH remained elevated during the seasonal anestrus, while it decreased in those kept with control bucks (Delgadillo et al., 2020). These findings indicate that, as in the "short-term male effect, in the "long-term male effect", the sexually active bucks also neutralize the negative feedback of estradiol on LH during the seasonal anestrous goats once they start their sexual activity after the end of the long days.

The buck-to-buck effect

As in female goats, in bucks, the sociosexual interactions can also break the natural inhibition of sexual activity during the seasonal sexual rest.

The "buck-to-buck effect" stimulates bucks in sexual rest

The introduction of a buck into a group of other bucks in sexual rest can stimulate the secretion of LH and testosterone, as well as their spermatogenetic activity and sexual behavior. This is what we called the "buck-to-buck effect". The response of bucks to the "buck-to-buck effect" depends of the intensity of sexual behavior displayed by the stimulatory males. Indeed, exposure of bucks in sexual rest to bucks made sexually active by a photoperiodic treatment induced an immediate increase in plasma LH and testosterone concentrations. By contrast, exposure to sexuallty inactive bucks did not induce any significant increase in these two hormones (Figure 3; Delgadillo et al., 2022). Thus, the "buck-to-buck effect" strongly resembles the classic "short-term male effect" that is observed in female goats. As in females, the "buck-to-buck effect" immediately and effectively "bypasses" the seasonal inhibition of the photoperiod on the central nervous system. This stimulation leads to a resumption of sexual activity of bucks during the seasonal sexual rest, which can be interesting on a practical level.

"The buck-to-buck effect" is maintained as long as the sexually active inducers males are active

In bucks joined with sexually active males, plasma testosterone remained significantly higher for at least 30 consecutive days more than in those joined with control bucks (Figure 4). In the same way, sexual behavior of bucks joined with sexually active bucks is higher than that of males joined with control bucks and remains of the same order as that of males that received previously the photoperiod treatment (Delgadillo et al., 2022). It is likely that the sexual activity of stimulated bucks gradually stops because that of inducing bucks does as well. However, it is interesting to note that the sexual behavior of stimulated bucks begins to be stimulated from the first day after contact with sexually active males, undoubtedly under the influence of testosterone, which also increases from 6 hours later and, perhaps also under the influence of other hypothalamic hormones.

Males induced by the sexually active males are also effective for a classic "short-term male effect"

Considering the previous results, we wonder if bucks stimulated by the "buck-to-buck effect" could be able to induce sexual activity in seasonal anestrus goats through the "short-term male effect"? In April, one group of anestrous goats was joined with the bucks stimulated by the "bucks-to-buck effect", while another group was joined with

bucks made sexually active by a photoperiodic treatment. Interestingly, the bucks stimulated by the "buck-to-buck effect" were as effective as bucks made sexually active by the photoperiodic treatment in inducing high ovulatory and estrous activities, leading to fertility which were not significantly different from one group to another (Fertility: 80 % vs. 85 %, respectively; Delgadillo et al., 2022).

Conclusions

The "short-term male effect" allows to induce and synchronize the sexual activity in anestrous goats. The "long-term male effect" also allows to induce and synchronize the sexual activity of goats during the seasonal anestrous, and allows goats to ovulate throughout the year. Finally, the "buck-to-buck effect" stimulate the endocrine and sexual activities of bucks during the seasonal sexual rest. Among the key factors in the success of these three biosexual stimulations, it is undoubtedly the use of the sexual active bucks which has attracted the attention and this is certainly what must be the subject of precise recommendations to breeders. The sexually active bucks reduce or overcome the negative feedback of estradiol and testosterone on LH secetion, by “bypassing” the very strong seasonal inhibition of the photoperiod on the hypothalamic-pituitary-gonadal axis. Altogether, these findings show the power of sociosexual relationships in controlling the seasonal reproduction of caprine species and it rebalances the relative weights of these relationships, in relation to the photoperiod, in the final control of seasonal reproduction during the year.

Keywords: Caprine, Reproductive seasonality, Photoperiod, Male effect, Buck-to-buck-effect.

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Figure legends

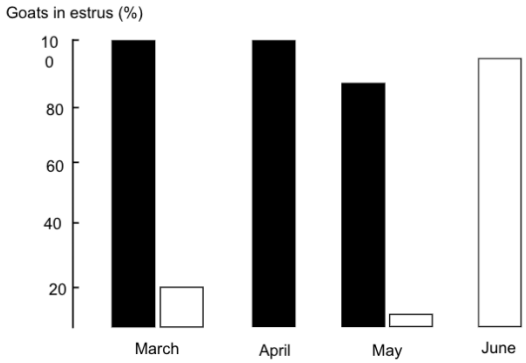

Figure 1. Goats displaying estrus when joined with bucks made sexually active (■) by photoperiodic treatments or untreated, sexually inactive bucks (□). In June, at the beginning of the natural sexual season, the untreated bucks displayed intense sexualy behavior allowing them to induce most goats in estrus (Delgadillo et al., 2011).

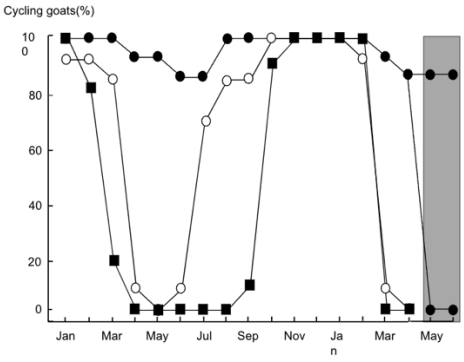

Figure 2. Ovulatory activity of goats isolated from bucks (■) or in permanent contact with vasectomized control bucks kept under natural variations of photoperiod ( ) or with those made sexually active by photoperiodic tretaments (●) during the seasonal sexual rest. Most goats joined with the sexually active bucks ovulated throughout the year. The grey area indicates when bucks were removed from half of the cycling goats; the ovulatory activity stopped in isolated goats (Delgadillo et al., 2015).

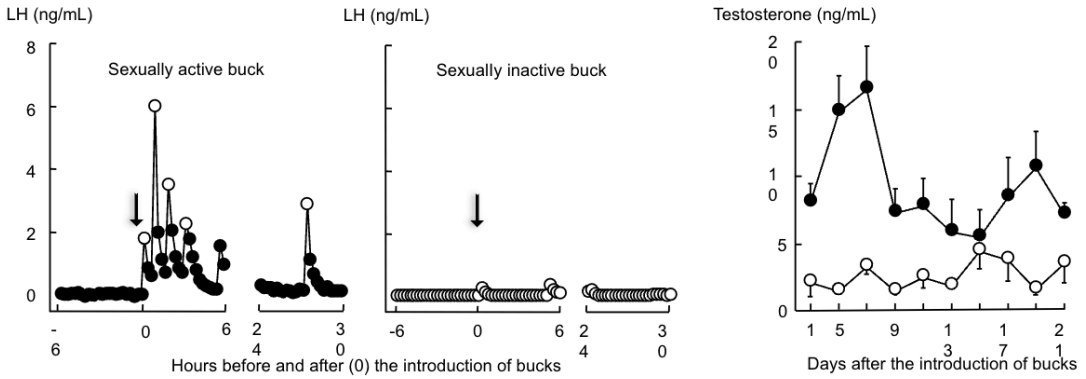

Figure 3. Individual example of LH pulsatility and testosterone concentrations (mean ± SEM) in bucks joined during the seasonal sexual rest with bucks made sexually active (●) by photoperiodic treatments or untreated, sexually active bucks (○). The arrows indicate the introduction of bucks; both hormones increased after the introduction of the sexually active bucks (Delgadillo et al., 2022).

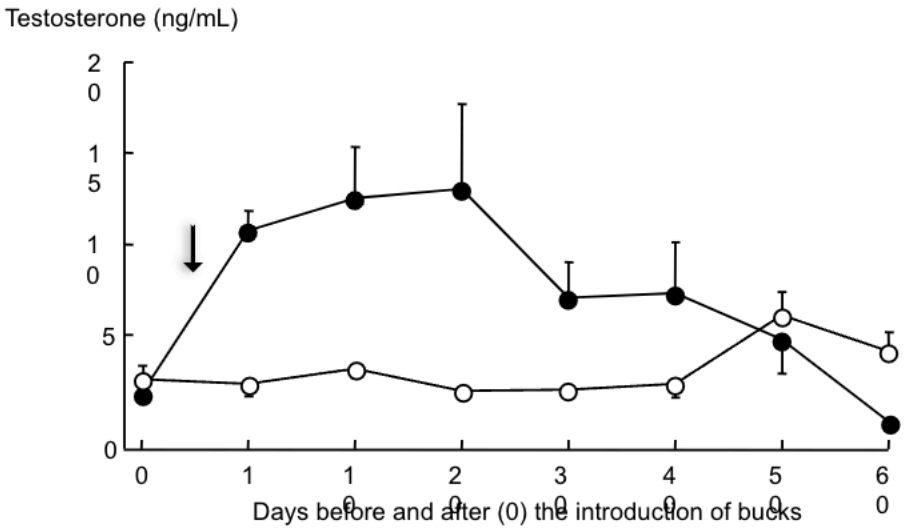

Figure 4. Long-term plasma testosterone concentrations (mean ± SEM) in bucks joined during the seasonal sexual rest with bucks made sexually active (●) by photoperiodic treatments or untreated, sexually active bucks (○). The arrow indicates the introduction of the bucks (Delgadillo et al., 2022).

Assisted Reproductive Technologies (ARTs) in sheep

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INTRODUCTION

Reproductive biotechnologies, also known as Assisted Reproductive Techniques (ARTs), serve as invaluable tools for enhancing the reproductive performance of animals targeted for breeding, thereby increasing the yield of offspring from these individuals. They also enable the generation of new and improved animals, or even add new attributes that did not exist in that species before. These technologies range from estrous synchronization and artificial insemination to the production of animals through genetic engineering. Through their application, in the past significant progress has been made in the genetic improvement of the ovine species, and more recently, more disruptive advances have been achieved with the use of cloning, transgenesis, and genome editing. Before the advent of these technologies, it has been necessary to understand various basic aspects of reproductive physiology and embryonic development, molecular biology, and genetic engineering. We present schematically the main novelties reported in recent years, with emphasis on the results obtained in our laboratory.

FIXED-TIME ARTIFICIAL INSEMINATION (FTAI).

It has been clearly established that follicular waves exist in sheep, under a similar pattern to what occurs in cows, goats, and other species. Antral follicles larger than 2 or 3 mm in diameter exhibit a wave-like growth pattern, which is determined by the endocrine system through the action of gonadotropic hormones (FSH and LH) and steroids (progesterone and estradiol). Although there is some variation in the number of waves that occur in each estrous cycle, the most frequent pattern in sheep consist of 3 or 4 waves per cycle. Additionally, follicular waves in sheep have been described during anestrus and early gestation (reviewed in sheep by Evans, 2003). Some of the most frequently observed characteristics in sheep are: 1) In each wave, at least one follicle with a diameter ≥5 mm is observed; 2) the largest follicle in each wave grows for approximately 5-7 days, with a growth rate close to 1 mm/day; 3) the maximum diameter reached by the largest follicle in a wave differs between waves; 4) as the luteal phase progresses and progesterone concentrations increase, the maximum diameter reached by the largest follicle decreases, follicular turnover is promoted, and the intervals between waves are shorter than during the early luteal phase; 5) during the mid-late luteal phase, it has been proposed that follicles that do not grow beyond 4 mm are not part of the wave phenomenon, suggesting that they represent a dynamic basal pool; 6) in most cases, the follicles that ultimately ovulate are those with the largest diameter on the day luteolysis occurs; 7) when double ovulations occur, the ovulatory follicles in most cases come from the same follicular wave, although they can also be part of different waves; and 8) double ovulations occur within a time range generally less than 12 hours. These and other aspects in follicular wave dynamics in small ruminants have been reviewed in depth by Menchaca & Rubianes (2004).

Follicular waves described above, particularly follicular recruitment and dominance, have a significant effect on the female's response to estrous synchronization treatments and superovulation, as well as on embryo production both in vivo and in vitro. For this reason, based on a better understanding of follicular dynamics, new protocols have been designed to better synchronize ovulation and achieve acceptable pregnancy rates with a single insemination without detecting estrus (fixed-time artificial insemination, or FTAI). The most recent treatments for FTAI better control follicular dynamics than conventional treatments, mainly by reducing progesterone exposure from 10-14 days (Long protocols) to no more than 5-7 days (Short-term protocols). These FTAI protocols were the result of the contribution of several studies and involve the use of an intravaginal progesterone device for 5-7 days combined with a dose of equine chorionic gonadotropin (eCG) and prostaglandin (PG) F2alpha at the time of device removal. Artificial insemination is performed a prefixed time without detecting estrus, between 48 and 56 hours after progesterone device removal. Different studies, in both sheep and goats, demonstrate that these short protocols are more convenient than long ones, allowing greater or at least similar results (never lower) than long treatments (reviewed by Menchaca & Rubianes, 2004).

Recently, we have generated new information - in addition to existing data - in large-scale FTAI programs comparing Short-term protocols (6 days) with Long-term prootocols (14 days). In all studies, we used intravaginal devices with 0.3 g of progesterone (DICO, Syntex, Argentina). In a first experiment, 1,750 multiparous ewes received intrauterine insemination via laparoscopy after Short-term and Long-term protocols. Pregnancy rate was significantly higher with the short treatmen than with the long treatment (43.5% vs. 37.8%, respectively; $P < 0.05$). In a subsequent experiment, it was compared the pregnancy rate achieved with a treatment with high progesterone levels for a short period of time versus low progesterone levels for a long period of time. A total of 922 ewes received FTAI using fresh semen via cervical insemination, after a treatment for 6 days (with first-use devices) or for 14 days with second-use intravaginal devices (previously used for 6 days). This experimental design generated two contrasting situations: one with high progesterone levels for a short period and another with low progesterone levels for a long period. Once again, the pregnancy rate was higher with the Short-term treatment than with the Long-term treatment (41.2% vs. 29.1%, respectively; $P < 0.05$), confirming the need to maintain adequate progesterone levels during the protocol. Overall, this information adds more evidence to the concept that as progesterone levels decrease in those longer treatments, certain conditions are generated that lead to lower fertility.

Additionally, applying these Short-term protocols of 6 days, in some cases, it is possible to reuse the devices at least once. The intravaginal devices containing 0.3 g of progesterone were designed for use in longer protocols of 12 to 14 days, so when using them for only 6 days, it is reasonable to consider a second use. However, it is necessary to take into account the concept stated above that low concentrations of progesterone during treatment negatively affect fertility, casting doubt on the effectiveness of using devices more than once. To elucidate this subject, we conducted several experiments in sheep (Vilariño et al., 2010; Vilariño et al., 2013; dos Santos Neto et al., 2015a) and goats

(Vilariño et al., 2011). When we used a CIDR device foe 6 days, we found that a second-use device, although it induces lower circulatory progesterone concentrations, allows for a similar ovarian response than a new first-use device. This acceptable ovarian response occurs even when the CIDR is used three times. However, these studies also showed that the pregnancy rate was significantly lower with devices used for the third time versus new devices, while the result was intermediate for second-use devices. This lower pregnancy rate in three-use devices was associated with a lower progesterone concentrations and lower follicular turnover. We suggest then that reused devices may yield acceptable results when used twice, and lower fertility with three o more uses would be associated - at least in part - with problems in follicular dynamics and the normal functioning of follicular waves.

In summary, various studies reported over the past 15 years demonstrate that Short-term protocols offer several advantages compared to longer protocols previously used, at least when using a progesterone device such as CIDR. The advantages can be summarized as follows: better control of follicular response and ovulation, acceptable fertility rates (generally higher and never lower than conventional treatments), a shorter period length required for programs implementation which practical implications in certain cases, and the potential for device reuse reducing treatment costs. Overall, these advantages in protocols facilitate increased adoption of artificial insemination, allowing this technology to have a greater impact on the genetic improvement of the flock.

SUPEROVULATION AND EMBRYO TRANSFER.

The better understanding of follicular dynamics mentioned above has also led to the development of new ovarian superstimulation treatments for embryo production and transfer. Currently, there are new alternatives for synchronizing the emergence of a follicular wave to achieve a better superovulatory response, utilizing the recruitment that normally occurs at the onset of the wave, thus avoiding the adverse effect of follicular dominance. One of the most innovative treatments is the Day 0 Protocol or Wave 1 superovulation treatment, successfully used in both experimental and commercial embryo production programs (Menchaca et al., 2009; 2010). This protocol involves initiating FSH treatment at the moment of the emergence of the first follicular wave (soon after ovulation, i.e., Day 0 of the cycle). To achieve this condition, ovulation must be synchronized beforehand, which can be done using the Short-term treatment described in the previous section. After that, ovarian superstimulation begin by FSH administration 72-84 hours after device removal, coinciding with ovulation and the emergence of Wave 1. FSH administration typically consists of 6 or 8 decreasing doses administered approximately every 12 hours. After superstimulating Wave 1, luteolysis is induced with two doses of PGF2alpha administered with the last two doses of FSH. Subsequently, ovulation is synchronized with a dose of GnRH 24 hours after the first PGF2alpha dose, followed by insemination 16 and 24 hours after GnRH. This treatment has improved embryo production compared to traditional treatments, both in sheep and goats (reviewed by Menchaca et al., 2010).

In a following study, it was demonstrated in sheep the benefits of exposing the oocyte to high concentrations of progesterone prior to maturation and meiosis resumption (Cuadro et al., 2018; Menchaca et al., 2018). For this reason, we have incorporated into the Day 0 Protocol the treatment with an intravaginal progesterone device during the

administration of FSH (from the first FSH dose to the first PGF2alpha). Ewes treated with progesterone had a higher fertilization rate (93.3% vs. 83.3%), greater number of transferable embryos per donor (5.4 ± 0.6 vs. 3.0 ± 0.7), and higher percentage of good-quality embryos (67.7% vs. 52.7%) than those that did not receive progesterone, respectively ($P < 0.05$). In addition, exposure of the preovulatory follicle to progesterone significantly improved oocyte quality (Menchaca et al., 2018). Overall, the results confirm the benefit of developing preovulatory follicles and oocytes with high concentrations of progesterone. Based on these findings, this strategy has been incorporated into the Wave 1 protocol with a CIDR inserted from the first to seventh FSH dose (in an eighth-FSH regimen dose).

While Wave 1 superovulation protocols like Day 0 protocol are effective and, in our experience, yield better results than the conventional approaches, many veterinarians continue to use traditional protocols as they have been the routine procedure for over 30 years. These protocols were developed in the 1980s and are deeply ingrained in daily practice, especially among veterinarians who began working several years before the latest concepts about follicular dynamics and ovarian superstimulation were described. For this reason, previous to develop the Wave 1 superovulation protocols we have attempted to refine these conventional protocols. Traditional protocols typically involve administering FSH to stimulate follicle numbers after 14 days of exposure to exogenous progesterone. Progesterone is administered using a silicone intravaginal device such as CIDR, and FSH is injected intramuscularly in 6 to 8 doses, usually starting 48 to 60 hours before removing the CIDR. In an experiment involving 239 donor ewes, we compared the duration of progesterone treatment to determine if such prolonged protocols were necessary. Progesterone treatment was administered using CIDR-G (0.3 g of progesterone, Zoetis) for 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days (23 to 25 donors in each experimental group). The results showed no significant differences in any of the evaluated variables, and embryo production was similar, demonstrating that such prolonged treatment is not necessary. In a second experiment involving 264 donors, we evaluated the use of eCG associated with FSH treatment, demonstrating that adding a dose of eCG to FSH treatment (at the time of CIDR removal) is not recommended since embryo yield is impaired. In another experiment involving 161 donors, we demonstrated the convenience of adding a dose of GnRH 24 hours after removing the CIDR. In summary, while traditional protocols have been shown to induce fewer or lower-quality embryos than Wave 1 protocols, if using such treatments, it is recommended to administer progesterone for 5 to 14 days, avoid using eCG along with FSH, and administer a dose of GnRH 24 hours after removing the progesterone device (Menchaca et al., 2009). However, when comparing this improved conventional protocol with the Day 0 Protocol, better results were obtained with the latter treatment (reviewed by Menchaca et al., 2010).

IN VITRO EMBRYO PRODUCTION (IVEP)

This technology maximizes the reproductive ability of a female by increasing the use of the ovarian follicular reserve. This allows for the frequent collection of oocytes from each sheep over extended periods, even when they are not normally ovulating (before puberty, during anestrus, or during gestation). Improved understanding of oocyte and

embryo development has allowed us to enhance this technology and achieve results applicable in commercial production conditions.

Laparoscopic ovum pick-up (LOPU) is the gold standard technique to aspirate ovarian follicles in commercial operations for IVEP in small ruminants. Previous to LOPU, follicular stimulation treatments are administered, and in general, females are synchronized using a progestagen during 10 to 12 days, 36 hours before LOPU a dose of FSH is administered, and the device is removed at the time of LOPU. After comparing different protocols, Baldasarre & Karatzas (2004) recommend a single dose of 80 mg of pFSH + 300 IU of eCG 36 hours before the aspiration. This is the treatment of choice for both sheep and goats. Novel alternative treatments have been recently evaluated, which take into account follicular dynamics and consider recruitment and dominance (Menchaca et al., 2018). Different studies in sheep and goats have shown that oocytes obtained by follicular aspiration and matured in vitro (IVM) have lower competence compared to those matured *in vivo* (Cognié et al., 2003), suggesting inadequate IVM conditions but also due to lower oocyte quality obtained by aspiration. Oocyte competence is affected by the size and developmental stage of the follicle from which the cumulus-oocyte complex (COC) is collected (Crozet et al., 1995). This is determined by follicular dynamics and dominance, extensively studied in cattle (Adams et al., 2008). The ideal method for aspirating high-quality oocytes should ensure a homogeneous population of medium-sized follicles (~4 mm), in growth or static phase, and containing healthy oocytes. The Day 0 Protocol described for superstimulation of the first follicular wave, may be applied also for LOPU. In this case, a single dose of 80-100 mg of pFSH is administered on Day 0 (i.e., 84 hours after removing the device when ovulation has already occurred). FSH was evaluated when reconstituted in MAP-5 (Vetoquinol, France) that contains hyaluronic acid allowing for slow hormone release. In this study, LOPU was performed 72 hours after the FSH dose (on Day 3 of the cycle). The results showed a higher number of aspirated follicles and better oocyte quality (Menchaca et al., 2018), encouraging further studies on this new approach to address this limitation and improve the quality of oocytes intended for IVEP.

Once the oocytes are collected from the follicles, they are subjected to maturation, fertilization, and in vitro culture under specific conditions of temperature, humidity, gas concentration, nutrients, hormones, and growth factors. There is a wide variety of media used by different groups that differ in their components, concentration, or procedures. The details used in our laboratory are described in depth in Menchaca et al., 2016a. After 6 days of in vitro culture, sheep embryos reach the blastocyst stage and can be transferred to recipients or cryopreserved for later use. Results with this methodology applied in our laboratory on data accumulated from 25,335 processed oocytes show a viable oocyte rate of 64%, a cleavage rate at 48 hours close to 80%, with subsequent blastocyst development varying between 30 and 50%. The pregnancy rate we have obtained after transferring fresh embryos on Day 6 of development generally ranges between 40 and 50%.

Low survival rate after freezing/thawing of in vitro-produced embryos remains as a limiting factor for this technology. When comparing fertility with embryos subjected to conventional freezing with ethylene glycol, we obtained a 7% versus a 46% pregnancy rate with in vitro-produced and in vivo-derived embryos, respectively (dos Santos-Neto et al., 2017). Slow freezing was developed for embryos produced in vivo; however,

embryos produced in vitro have some intrinsic differences that negatively affect their cryotolerance, which is associated with excessive lipid accumulation, metabolism alteration, changes in structural and physical embryo characteristics, among others. As alternative, vitrification methods for in vitro-produced embryos have been studied in different species and is routinely applied in human assisted reproduction, allowing successful cryopreservation of oocytes and embryos. In small ruminants, several studies have been conducted to test different embryo vitrification methods. Overall, the efficacy of the vitrification technique depends on various factors besides the species, such as the embryo's developmental stage, the embryo's origin (in vivo or in vitro), the volume to be vitrified, the cooling rate, cryoprotective media, among others. The outcomes following the vitrification of in vitro-produced embryos, especially concerning embryo survival and pregnancy rates, have been at best, marginally acceptable in sheep and goats (20 to 30%, Cognie et al., 2004).

New vitrification methods have emerged that involve using a minimal volume of medium (approximately 0.1 µL) in which the embryo is placed for vitrification, allowing for even faster vitrification than previous vitrification systems. The most well-known method utilizing this ultra-rapid vitrification strategy and minimal volume is the Cryotop (Kuwayama et al., 2007), which we have successfully used in our laboratory (dos Santos-Neto et al., 2015b). In a recent experiment conducted in sheep (Menchaca et al., 2016a; dos Santos-Neto et al., 2017), the pregnancy rate was higher when vitrification was performed by the Cryotop method (77.8 and 55.1%) than by conventional freezing (64.9 and 11.1%), for in vivo-derived and in vitro-produced embryos, respectively. Therefore, at least in our production system, the Cryotop method allows promising results, which could favor the implementation of programs for transferring in vitro-produced embryos in production species.

GENOME EDITING BY ARTs.

The production of embryos in the laboratory represents an extremely powerful tool not only for classical genetic improvement but also for the development of new biotechnologies. Embryo micromanipulation enables the use of the revolutionary CRISPR/Cas system for genome editing technology. With this cutting-edge tool, it becomes feasible to modify, eliminate, silence, or incorporate DNA sequences at early embryo stages like never before, resulting in animals with more desirable characteristics for various purposes (Menchaca et al., 2016b). As an example, through the application of this technology we have successfully induced the suppression of *MSTN* gene expression in sheep. This gene codifies for myostatin, a protein associated to muscle development and differentiation, and by the intervention and silencing of this gene we obtained double-muscled animals. Implemented in superfine Merino sheep embryos, a breed specialized in very high-quality wool, it was achieved dual purpose lambs producing both high quality wool and more meat (Crispo et al., 2015b). The CRISPR-Cas system involves designing an RNA guide identical to the DNA sequence to be modified; thus, once injected into the zygote, this construct precisely recognizes, through complementary base pairing rules, the corresponding sequence in the DNA. Additionally, this guide RNA is associated with an endonuclease (Cas9) that acts as a molecular scissor and cuts the DNA at the precise site directed by the injected guide RNA. Once the double-stranded DNA is cut, the repair process that normally occurs can

proceed in two ways: a) by deleting or replacing nucleotides in this portion, thereby rendering the gene non-functional and the protein it coded for absent in the new individual (generating a knock-out animal, KO); or b) by inserting a pre-established sequence that will give rise to a known function, for example, to correct a gene or add a new protein in the individual (knock-in animal).

We have also generated other KO models using CRISPR-Cas technology in sheep to study more fundamental aspects of certain endogenous proteins using ovine models. Similar advances have been reported in goats, while the first report in cattle was some years later (Gao et al., 2017). In summary, the development of molecular biology and genetic engineering has allowed significant advances in genome editing in recent years. Reproductive biotechnologies have made it possible to use this knowledge to generate embryos and animals with certain genome improvements, and to incorporate or enhance characteristics of productive interest in traditional species. Likewise, it is possible to generate animals as bioreactors for the production of drugs in their milk or even to advance in medical therapies and xenotransplantation, much more precisely, quickly, easily, and economically than with classical transgenesis.

CONCLUSION

The understanding of ovarian physiology and embryo development has contributed to enhancing or even developing new technological tools applied to ruminant reproduction. This makes it possible to significantly multiply the genetics of those individuals of interest, closer to their maximum reproductive potential. Furthermore, it allows for the generation of new animals with edited genomes according to our interests. Many of these biotechnologies are now available worldwide, while others, more modern ones, have not yet arrived to the productive sector and are still in a developmental phase. All of them represent an important contribution in different fields: some contribute to livestock production on a small or large scale, while others are used in more basic areas of science or even in biomedical or pharmaceutical applications.

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Buffalo and camelids

South American camelids in society and research – an update

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Introduction

The keynote lecture on South American camelids (SAC) will give a short introduction to the animals for all veterinarians attending the WBC which have no or minimal experience with SAC. It will further focus on changing use of domesticated SAC in developed and urban areas and recent developments in research on the SAC.

South American camelids include the domesticated species Llama and Alpaca and the non domesticated species Vicuna and Guanaco. The term New World camelids is also used as synonym for SAC. The Old World camelids (Dromedary and Bactrian camel) and New World camelids have common ancestors but they are developed separately over about 50 million years either in Asia or South America.

Anatomical and physiological features

All camelids share numerous anatomical and physiological features; however, the long time of separate development resulted in some differences. Camelids are taxonomically not classified as ruminants although the gastrointestinal tract has some similarities to ruminants. Camelids have a first stomach (compartment 1) similar to the rumen and they are chewing the cud resulting to the same ability like ruminants to digest plant fiber which is indigestible for monogastric animals. One major difference to the ruminants (ungulates) is that camelids do not have claws covering the distal phalanx of the limbs but toenails dorsally and a fast-growing epithelium covering connective tissue and fat ventrally forming the sole (tylopods). Since SAC are living in high altitudes they are adapted to the low oxygen pressure by having hemoglobin with a high oxygen binding capacity.

Use of SAC

Originally SAC were solely kept in South America, where they have been used for fiber production (mainly alpaca), transportation (mainly llama), and meat production over centuries. Until about 40 years ago only few animals were kept outside of South America mainly in zoos; however, beginning in the 1980ies keeping and breeding of llamas but especially alpacas started in North America, Australia and New Zealand as well as in Europe. In Europa SAC were initially introduced in the UK, the Netherlands, Germany, Switzerland, and Austria but meanwhile SAC herds can be found in many other European countries.

After introduction in the new countries the animals were continuously used tor fiber production, however traditionally uses in the South America like meat production or transportation are of low or no importance. However, new areas using SAC have been developed and are still developing. SAC are frequently used for leisure activities like

walking, which may vary from short walks of one hour to strenuous alpine tours over several days. Other activities are photo shootings for wedding ceremonies, presentation of animals at fairs or markets, open farm days, visits of schools and kindergartens and more "fancy" activity as "blind-dating with alpacas", "moon light walks" or "alpaca yoga". It seems that the creativity of the owners seems endless generating new business ideas. Animal supported therapeutic activities for humans are different areas of SAC use which are developing rapidly. SACs are used in therapeutic activities for rehabilitation, in people with mental health problems, autistic people or people with dementia. These new activates resulted in concerns according to biosecurity for the people who often might be especially vulnerable but also in welfare concerns since SAC are put in situations (e.g. frequents transportation, use in buildings with unsuitable floors, noise, isolation from the
herd) which may cause massive stress for them. Research work is needed to assess these issues and support the owners to avoid stressful situations for the animal and guarantee animal welfare.

Veterinary care

A logical consequence of the wider distribution of SAC worldwide was the increasing demand for specialized veterinary care. However, till the end of the last century SACs were not part of the ordinary curriculum of the North American, Australasia or European vet schools. Meanwhile the situation has generally changed; the majority of the vet schools in areas where SAC are kept have integrated the SAC in their teaching. Additionally, courses have been offered in many countries to transfer knowledge and skills to veterinarian who graduates before. Veterinary groups or associations are available for veterinarians who are interested in SAC like the British Veterinary Camelid Society, the Section for Small Ruminants and SAC within the German Veterinary Association or the Austrian Buiatric Association which included SAC. There are specific national but also international conferences on SAC, but SAC topics are also increasingly included in larger congresses like the WBC or the Leipziger Tierärzte Kongress the major multispecies and multitopic veterinary congress in Germany with about 7000 attendees.

Additionally, since the SAC were only regionally kept there was only limited generally accepted knowledge available. Meanwhile several well accepted textbooks are available in different languages (see reference list below) but there is still lack of knowledge in some areas like the mostly unknown pharmacology in SAC which results that numerous drugs are used applying dosages derived for other species.

Research

Traditionally research in SAC in South America was focused on reproduction resulting in a massive increase of knowledge in these areas. Later the research activities were extended to other scientific areas when research on SAC started in North America, Australasia, and Europe. The number of published peer-reviewed research papers has been increased substantially from 1970ies (about (10 to 20 publications per year) to over 250 in 2023. Nevertheless, research in SAC has still to be considered a niche in comparison to other animal species.

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Public health, food quality and traceability

Key Aspects to Ensure Silage Quality

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The quality of silage is complex to define. We can refer to quality when considering the nutritional composition of the silage, but also when evaluating the fermentative characteristics of the process. On the other hand, we can refer to quality in terms of safety or how healthy the material is for the animals. The truth is that all the aforementioned characteristics are affected in one way or another by many factors, but there are 6 factors that undoubtedly affect quality no matter how we analyze it.

Before analyzing the factors and basic concepts that affect the quality of silage, we must define what silage is and why it is an important tool in the nutrition of farm animals.

The phenological nature of plants, as well as climatic seasonality and the fluctuation of prices for fertilizers and other inputs necessary in animal production, necessitate livestock producers to plan for food production. This is especially crucial for forage resources, which are not only essential for production but also for maintaining the health of animals, particularly ruminants, who are physiologically dependent on fiber.

Silage is defined as the process by which materials, especially forages, are preserved through lactic acid fermentation, which results in the material being pickled to a sufficiently low pH to prevent a range of decomposing microorganisms from consuming its nutrients and initiating undesirable fermentation pathways.

This lactic acid fermentation is achieved when a series of general conditions are met, such as the presence of soluble sugars, an anaerobic environment (absence of oxygen), and suitable microorganisms. If these conditions are met, the producer can preserve the nutritional characteristics of fresh forage over time. This enables them to better cope with periods of scarcity, standardize production throughout the year, and reduce dependency on seasonal fluctuations in feed prices for their animals.

For this reason, this conference aims to clearly and simply present the main six factors that can affect the quality of ensiled forage and that can be controlled by producers.

Optimal Harvest Point: Maturity and Dry Matter Level.

Nutrients in feed are concentrated in the dry matter fraction, as water is H2O. Therefore, it cannot be assumed that protein, carbohydrates, lipids, minerals, and vitamins are present in the water fraction. Consequently, it can be deduced that a higher dry matter content allows the animal to consume more nutrients. On the other hand, excess moisture in the feed to be ensiled can affect the ensiling process, altering the type of fermentation generated and consequently the quality of the silage.

Defining the optimal dry matter content for ensiling can help reduce losses due to effluent, as a significant portion of the nutrients and acids formed in the process can be lost when the material moisture is high, leading to seepage during compaction and storage.

The minimum dry matter content required for ensiling is 30%. For moisture levels below this threshold, it is recommended to use the pre-drying technique. It's important to consider that cellular respiration initiates the deterioration of forage. Additionally, using an absorbent material such as corn distillates or ground corn can help correct the moisture level.

Figure 1: Addition of Dry Corn Distillers to Correct Excess Moisture in Forage and Improve Fermentation Conditions.

Another way to modify the dry matter content of certain forages is through crop age or maturity level. In general, grasses and cereals increase their dry matter concentration with crop age. Therefore, it is recommended to measure the increase in dry matter and the maturity level of the grains.

For maize, an optimal dry matter range for ensiling is typically between 32% and 38%. The correct method to determine this is through drying using an air heating oven. However, resources may not always be available to perform such analysis. In such cases, evaluating the milk line of the grain is a practical tool with good predictive ability. It's worth noting that there may be variation in predicting dry matter content based on the advancement of the milk line with certain hybrids.

Figure 2: Identification of Milk Line Progression to Establish an Optimal Harvest Point Based on Plant Maturity.

The concept suggests that as the grain matures, it becomes harder from the outside inward and concentrates the level of starch and thus the dry matter. In some hybrids, there is a correlation between dry matter and the percentage of starch it contains. Therefore, we can estimate that when maize has 35% dry matter, its starch percentage ranges from 33% to 35%.

Una correcta materia seca a la hora de ensilar va determinar mejores condiciones de fermentación, composición nutricional y palatabilidad lo que produce mejores consumos y por ende mayor producción y salud de hato.

Dry Matter %	Starch %	NDF %	Crude Protein %	ME Mcal/kg DM	pH
< 20	10.3	57.0	8.78	2.27	4.45
20 - 25	20.8	53.7	8.41	2.39	3.91
25 - 30	28.0	48.2	7.68	2.53	3.79
30 - 35	31.8	46.0	7.13	2.54	3.78
> 35	34.2	44.9	6.95	2.54	3.84

Table 1: Relationship between Dry Matter Content and Nutritional Composition and pH Achieved in the Silage Process of Corn Forage.

Table 1 demonstrates that as the dry matter content improves, the pH decreases to indicated levels, and the levels of metabolizable energy, neutral detergent fiber, and starch are better, showing that dry matter content is crucial when considering silage quality.

Cutting Length and Cutting Height

To understand the importance of these two characteristics, it's worth mentioning that they are adjusted by manipulating harvesting machinery. The first premise to clarify is that a smaller particle size increases the surface area of contact (colonization points) for bacteria to initiate the fermentation of plant sugars. Another point is that a higher chopping length will physically increase the material density, making the oxygen extraction process more efficient and resulting in higher silage quality.

On the other hand, excessive chopping can reduce the physically effective fiber of the forage and may lead to episodes of ruminal acidosis. For this reason, chopping lengths smaller than 6mm are not recommended. Particle size has a significant effect on reducing losses due to effluent, especially when the moisture level exceeds the recommended amount. Conversely, a very long particle size when the crop exceeds the recommended 38% dry matter can have negative effects on compaction efficiency, leading to undesirable butyric fermentations and the presence of alcoholic fermentations via yeast action.

The cutting sizes will vary with the percentage of dry matter of the forage at the time of harvesting and the type of machinery used. In regions where machinery lacks grain processors, such as tropical countries in America like southern Mexico, Central America, Ecuador, Colombia, and some parts of Brazil, the recommended particle size ranges from 6mm to 13mm. For machinery equipped with grain processors and appropriate dry matter content (around 35%), the cutting length is typically 19mm, which can be reduced to 15mm for very dry forages or increased up to 25mm for very wet materials.

The calibration of the grain processor varies from 1mm to 3mm depending on the hardness of the grains to be harvested and the type of machinery.

The effect of cutting height on the quality of the process and nutritional composition is based on two concepts:

Firstly, at a greater height, fewer weeds are harvested, which may contain a higher percentage of moisture and anti-nutritional compounds. Additionally, there is a lower probability of harvesting soil in case of uneven ground, which not only affects nutritional values but can also introduce contaminated organic matter into the silo.

Secondly, cutting height affects fiber levels and consequently the digestibility of the material. When harvested at a greater height from the ground, the woodier part of the stem, where support structures are concentrated, is left in the field. This includes NDF (negatively associated with voluntary intake) and ADF (negatively associated with energy value).

Correct Use of Inoculants

Inoculants are defined as biological products containing live and viable bacteria, sometimes combined with enzymes. These bacteria are responsible for fermenting the plant sugars, primarily producing lactic acid, to achieve the correct pH decrease and desired fermentation. Meanwhile, the enzymes function to break down or simplify complex carbohydrates into simpler ones and sugars, providing bacteria with more substrate for fermentation.

Inoculants nowadays are highly specific. We can find inoculants for different types of forages. It's not the same to ensile legumes as it is to ensile grasses or cereals; each has distinct fermentative characteristics. Likewise, climatic conditions and moisture levels of the material at the time of harvesting are important factors to consider when selecting the type of inoculant.

Basically, inoculants can be divided into two main groups: those containing homofermentative lactic acid-producing bacteria (LAB) and those containing heterofermentative LAB. This division is focused on addressing the main challenges of the material when being ensiled.

The first group is used in forages with low levels of dry matter, where there is a natural tendency to generate a certain amount of acetic acid. Therefore, it is not advisable to use an inoculant with heterofermentative bacteria, as a highly acidic silage can be expected.

On the other hand, when materials have dry matter above 36%, the risk of aerobic damage is higher, such as in high-moisture corn or cereal grains. In such cases, it is recommended to use a heterofermentative inoculant to exert an inhibitory effect on fungi and yeasts. This increases the stability of the silage once the silo is opened.

Apart from all the aforementioned, it's of utmost importance to analyze the indicated strains with their official identification numbers. Merely mentioning L. buchneri or Pediococcus pentosaceus is not sufficient, as there is a wealth of information available currently on each strain and its specificity. Therefore, one inoculant is not the same as another simply because they have the same names of bacteria and enzymes, and fermentation results can vary.

It's crucial to pay attention to the concentrations of colony-forming units per gram of inoculant when purchasing them, as this will be directly correlated with the dosage of product to be applied per ton and, consequently, with yield. The recommended and FDA-approved values for different inoculants, depending on the nature of the bacteria they contain, are:

Type of Inoculant	Minimum Count to be Applied
Homofermentative Bacteria	100 000 UF /g of forage
Lactobacillus buchneri 40788	400 000 UFC/g silage 600 000 UFC/g de HMC

Table 2: Minimum Application Values of CFU/g, Depending on the Type of Inoculant and Type of Material to be Silaged, as per FDA Guidelines.

In the case of enzymes, each one must also be declared, and a minimum concentration value must be guaranteed, matching the values reported as effective in various studies published on their use in ensiling processes.

Silo Compaction and Sealing

The proper extraction of air in ensiling is of paramount importance. The presence of oxygen influences the fermentative pathway that will be generated, as well as the presence of pathogenic microorganisms that not only limit nutritional quality and palatability but also affect the safety of the silage and the health of the animals fed.

Understanding this, the premise is that compaction should be carried out to eliminate residual oxygen as much as possible. Therefore, controlling the density of the silage is the most indicative parameter of packaging quality. Proper compaction not only keeps dry matter losses low but also optimizes the use of space required for storing the feed and reduces losses during extraction and feeding to the animals (feedout). The presence of oxygen leads to the proliferation of molds and yeasts. Evidence of such presence includes the smell of alcohol and high concentrations of ethanol in a laboratory analysis (above 3% of dry matter).

The parameters for assessing how well compaction and subsequent oxygen extraction are being performed are as follows: ideally, the density should be 350 kg of dry matter per cubic meter. Another more practical parameter is the degree of sinking that the boot generates when walking on the silo being prepared. Additionally, it is considered that when compaction is good, the tractor's footprint should only mark the tire tread and not the groove it passes through.

Correct Silo Sealing

While it's true that anaerobiosis in the silo is achieved through proper compaction of the material in each layer, another challenge is to maintain that anaerobiosis over time, and this is achieved with a correct closing and sealing of the silo.

Important factors such as the material used for sealing and the counterweights placed to ensure that the plastic does not lift and cause air pockets, leading to damage to the upper layers of the silo, are crucial. Currently, highly efficient technologies are available for sealing materials and silo coverage. Knowledge has advanced over time; initially, simply covering the silo with commercial plastic and adding weight was sufficient. Nowadays, barrier oxygen plastics are used, which are thin and are sought to be as adhesive as possible to the forage surface. There are plastics that are only oxygen barrier and others for coverage. Additionally, 2-in-1 plastics are common nowadays, which have around 7 layers containing some type of polyamide and ethyl vinyl alcohol in the middle, significantly increasing oxygen permeability.

Figure 3: Use of tires on the silage plastic to generate weight and reinforce sealing and airtightness during storage.

A silo sealing plastic or tarpaulin should be: resistant to sun exposure, as it can withstand the sun's rays; as adhesive as possible to the forage surface to prevent air storage; have good tear resistance; preferably white on the outside and green or black on the inside; and be covered or pressed with structures that serve as counterweights, such as old tires, preferably with cut profiles and tread sheets to prevent water retention and the proliferation of mosquitoes and pests. It's also common to use sandbags, soil, or wooden pieces or wheels that can be placed to exert weight on the cover. In the case of wooden pieces or wheels, it's necessary to prevent possible tears that may occur on the plastic.

Feedout Management

In this section, a series of very simple and common-sense practices are proposed. We start with the opening phase. The plastic should not be removed beyond the depth that will be harvested daily. For this, it's necessary to calculate correctly the amount of feed to be used and the density at which the silo was packed. By knowing the height and width, it's easy to calculate the depth that should be removed daily.

Once the silo is opened, it's not advisable to cover it again each day because this can lead to an exponential growth of molds and yeasts. Remember that these microorganisms have very short life cycles, so in one night, with abundant humidity, nutrients, and oxygen, we can find the presence of hyphae the next day.

The face of the silo should be kept tight and vertical to prevent an increase in the exposure surface to oxygen. Additionally, extracting the feed vertically will prevent the loss of compaction and increase the rate of oxygen penetration.

Figure 4: Correct Management of the Front Face of the Silo Once Opened for Feeding.

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Animal behavior and welfare

Stress and its Impact on Disease in Calves

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Although most people understand the word "stress," it can be difficult to define. However, it is basically the calf's physiologic or behavioral response to adverse events in the environment or management system. These responses allow the calf to adapt or cope with the adverse effects causing stress. Things like hot or cold weather, overcrowding, shipping, weaning, pain, poor nutrition, erratic feeding schedules and improper handling can all cause stress and make the calf more susceptible to disease. We are learning that stress has a profound effect on the immune system and can dramatically increase rates of disease. In the 1980's, a survey sent to beef producers in Western Canada identified the most common risk factors in their opinion for diarrhea outbreaks in the calves. The results indicated the greatest risk factors for calf diarrhea were inclement weather (cold), poor ground surface conditions (wet, cold pasture) and overcrowding; all factors that would increase stress levels in the calf.[21] Many heifer growers or veal operations will say that disease exposure is expected and constant. Despite the constant exposure, disease outbreaks seem to be sporadic - often centered around times of stress. Recent studies have even found that bacteria can detect stress in the animal and multiply during those periods. The principle is known as "quorum sensing" where pathogens living in the host at low levels can sense changes in norepinephrine (a hormone produced during stress) and begin to multiply. Although some stress is likely inevitable in raising beef or dairy calves, this should be kept to a minimum. The purpose of this presentation will be to discuss the different causes of stress that calves face and how we can minimize their impact in causing disease.

1) Nutrition- Adequate calf nutrition is critical for host immunity, and energy deprived calves are more likely to have increased morbidity and mortality due to diarrhea. There is a significant amount of new data indicating calves fed at a higher plane of nutrition are more resistant to disease as compared to calves on more conventional milk feeding programs. For example, in one study calves fed an accelerated growth milk program (28% protein, 20% fat) maintained hydration, had faster resolution of diarrhea, had increased body weight gain and better feed conversion after experimental challenge with Cryptosporidium parvum as compared to calves fed conventional milk replacer (20% protein, 20 fat).[20] A similar study done with a Salmonella Typhimurium challenge showed that not only were calves on a higher plane of milk nutrition more resistant to disease, but they had a more robust immune response to Salmonella challenge than calves on lower planes of nutrition.[1] A more recent study from Bavaria examined herd specific risk factors correlated with "high" and "low" risk of calf diarrhea on German dairy farms and found that being fed ad libitum milk in the first week of life was the most significant variable that reduced the risk of diarrhea.[17] While many people have long recognized there was a strong correlation between calf nutrition and rates of disease, we have only recently had data to illustrate the importance of this relationship. With the plethora of new studies published in the last 5 to 10 years, it has been hard to avoid the realization that we have been significantly underfeeding dairy heifers for a

long time and that higher planes of nutrition provide multiple benefits. Therefore "starvation" or calves in a negative energy balance is still one of the biggest stresses in dairy calves that we are working to overcome.

In addition to hungry calves, additional nutritional stresses include erratic feeding schedules and inconsistent meals. For example, some dairies choose to feed waste milk when they have it but switch to milk replacer and/or a combination of waste milk and powder when they don't have enough. This is also stressful and can contribute to diseases like diarrhea, abomasal bloat and abomasal ulcers.[3] It is recommended to start calves on milk replacer which is generally more consistent than waste milk for the first 2-3 weeks and then incorporate a "mix" of waste milk and powdered milk later in the feeding period. And maintaining a consistent osmolality (or total solids) is also important.

Lastly it is important to maintain calves on a positive plane of growth through the weaning period. We often see high rates of respiratory disease on dairies that have an aggressive step down (or no step-down) going from large volumes of milk (8 or more liters per day) to weaning (no milk) over a short period of time. Calves should be eating at least 2-3 kg of grain (starter) per day and should remain on the same starter for at least a week after moving to group pens to reduce the "stress" associated with a diet change. It takes energy to run an immune system. When calves go off feed shortly after being comingled, that is a great opportunity for pneumonia to start.

2) Weather- It's well accepted that heat and/or cold stress can be a significant factor contributing to disease in calves. In the early 1980's, veterinarians in Australia noted that calf diarrhea rates went way up during the summertime with histologic evidence of severe enteritis at necropsy.[19] A study done in Florida followed 3,300 calves from birth thru 14 months of age and found that season was a risk factor both for incidence of disease as well as growth rates.[9] A large epidemiologic study examined the risk of weather conditions on the occurrence of respiratory disease in feedlot cattle within a 45-day period after arrival.[6] The study included data from 1,904 truckloads of cattle (288,388 total animals) and 24,947 cases of pneumonia. The most significant weather factors associated with development of respiratory disease were change in temperature throughout the day and maximum wind speed. Although there is little we can do to control the weather, producers need to have a plan in place for controlling heat and/or cold stress depending on where they live. In addition, if they are aware of "higher risk" times of the year, farm employees can be sure they are detecting respiratory disease aggressively and potentially incorporating metaphylaxic antimicrobials in countries where this is legal to help minimize the occurrence of disease.

3) Housing- Available housing systems for dairy calves include individual, pair-housing, or group housing. Social rearing of calves is increasing in popularity due to consumer concerns and potential benefits which include improved growth, social interaction, and improved ability to adjust to novel environments.[8] There have been conflicting studies regarding the incidence of disease associated with group versus individual housing. Some studies show increased rates of disease with group housing, while others show no difference. There are also conflicting studies that show "hutch" type housing produces less disease than barns, but again there haven't been consistent results. It's likely that other factors such as space allowed, ventilation, group size, nutrition,

etc matter more than just the type of housing. For example, a recent study showed that calves housed in crates with 1.85 m^2 of living space had lower haptoglobin levels and consumed more starter than calves housed in identical crates with 1.23 m^2 of living space.[13] The calves with more space also had more robust immune responses to lipopolysaccharide (LPS) and phytohemagluglutinin-P. Certainly, when we utilize group housing, we need to manage the group size. For example, a study done in Sweden showed that calves housed in groups of 6 to 9 had lower pneumonia rates and better average daily gain as compared to calves housed in groups of 12-18.[24,25] Another study in the United States conducted in 38 different farms utilizing group housing and automated feeders showed that group size was highly correlated with disease rates.[15] They suggested group size be limited to no more than 15 calves.

4) Transportation- Very few dairy calves were transported in the first few weeks of life 25 years ago in North America, however it's no longer uncommon for calves to be trucked 12 hours or more now as animals go to specialized calf raising facilities with increasing frequency. In addition to heifers, in the United States, the majority of dairy bull or beef-dairy cross calves within the first week of life will be transported off-site to be marketed for meat and either go through an auction barn or be transported for long distances to their final destination. In 2014, the United States Department of Agriculture (USDA) National Animal Health Monitoring System (NAHMS) reported that 97.6% of operations sold bull calves prior to weaning at an average of 7.6 days of age (SD = 11.8 days, median = 4 days) with 48.6% of these bull calves sold through an auction barn.[22] Since calves being transported may likely be immunocompromised from poor colostrum intake, there is a greater mortality risk associated with the transportation process.[23] Multiple factors can impact the
welfare of calves but transportation time, feed withdrawal, and stocking density are the most critical factors influencing calf mortality during and after transport.[23] A study in Australia found that mortality rates in newborn dairy bull calves increased exponentially with distance of transportation to farms or to slaughter.[5] In beef calves, distance of transport has been strongly correlated with an increased risk of respiratory disease upon arrival to a feedlot.[7] In addition to transportation time, feed withdrawal can also have a significant effect on calf health and welfare. In calves transported more than 16 hours without feed, blood glucose levels dropped dramatically when compared to those calves traveling 12 hours or less.[10]

Physiologically, transportation impacts several aspects of the immune system that makes calves more likely to get respiratory disease. For example, ciliary function is inhibited by dehydration which is relatively common in calves hauled long distances.[4] This makes calves less capable of keeping viruses and bacteria that live in the "upper" respiratory tract out of the lungs. Transport also decreases the production of antibacterial proteins such as defensins and lactoferrin that also increases susceptibility to disease.[4] Transportation stress has been shown to have a direct and profound effect on the immune system and can alter neutrophil trafficking[2] as well as alveolar macrophage numbers.[14] Age at the time of transportation and its correlation to disease is a subject that needs more research. In many European countries, calves are not allowed to be transported in the first 2 weeks of life. However, in other countries (like the United States), dairy calves are often transported within the first few days after birth. A recent study done in Canada found that calves over 7

days of age had reduced risk of abnormal respiratory clinical scores compared to calves that were 2-6 days of age and transported equal distances.[11]

5) Ventilation- Studies have linked increased rates of respiratory disease to poor ventilation in calf housing.[16] We know for example that chronic exposure to high ammonia levels can disrupt ciliary function and degenerate the nasal epithelium making it more difficult to remove viruses and bacteria from the lungs.[4] Maintaining good air flow and air quality and the level of the calf is critical in all types of weather for maintain low airborne bacterial counts.

6) Weaning- Weaning is another common stress in both beef and dairy calves that can precipitate respiratory disease. In addition to taking the milk away, many calves experience other stresses around this time including a change in their feed (diet), a change in their housing type, social stress from a transition to group housing, vaccination and painful procedures such as castration and/or dehorning. Although weaning is a necessary part of the calf raising process, we need to take steps to minimize the amount of stress it causes. Things like keeping calves in their same environment (hutch, barn, pasture, etc) for a week or two after weaning, time vaccinations prior to weaning, limiting group sizes when moving out of the hutch and limiting transportation immediately after weaning can help minimize the stress associated with this process and help prevent disease. It's also critical to make sure calves continue on a positive plane of nutrition after weaning. When calves are weaned and immediately placed into large groups on a new diet, they often have a dramatic reduction in feed intake for a couple of days which is a great environment for a respiratory outbreak.

Lastly there is data to suggest that some calves are able to "handle" stress better than others. For example, a study done in Belgium looked at cortisol levels on arrival to a veal operation in Holstein calves.[18] Calves were 2-4 weeks of age and shipped to a single veal operation from different farms. After arrival, they were ultrasounded weekly for lung lesions. The results showed that cortisol levels varied widely in calves at the time of arrival, however calves with higher cortisol levels were more likely to develop chronic, non-responsive pneumonia. This suggests we may be able to eventually have the technology to select calves that are more capable of handling stress and thus less likely to get disease. Studies have been published validating the use of genomic markers to reduce calf diseases.[12] Perhaps these tests are largely selecting for animals that have an increased ability to handle stress and thus are more resistant to diarrhea and/or pneumonia.

Ultimately stress plays a significant role in the development of disease in both beef and dairy calves. Although some stresses (ie. weaning or bad weather) are inevitable, producers need to be aware of them and work to limit their impact.

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More energy for pregnant goats, improves mother-young behavior??

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Maternal behavior has been widely studied in laboratory animals as rabbits or rats as well as in the sheep (González-Mariscal, 2001; Bridges, 2015; Poindron and Le Neindre, 1980). Goats is a specie which little work has been performed about the environmental, sensory and physiological factors affecting the maternal behavior compared with the sheep. Similarly, because while sheep have been to possess a spatial mother-young of type “follower”, in the goat it has suggested that mother-young relationship is “hiding”. Therefore, it has been proposed that between both species show great differences in the mother-young relationship. Most studies in the sheep are performed under an optimal feeding schema, thus covering their nutritional requirements of the animals. However, in the grazing goats, which could be undernourished, limited information exists about the effects of nutrition on the characteristics of maternal behavior.

In the present, we communicate some results obtained in the Centro de Investigación en Reproducción Caprina (CIRCA) from the Universidad Autónoma Agraria Antonio Narro. The CIRCA is located in the Comarca Lagunera region at Mexico which exist near the 500,000 caprines. Most animals in this region are result of crosses of local animals with Alpine, Saanen, Toggenbourg and Anglo-Nubian breeds. Under this semi-desert area, a great proportion of the animals graze on the natural available vegetation which in turn is the result of the distribution and amount of rainfall throughout the year. Thus, the dry season in this part of northern Mexico lasts from December to May, which coincides with a dramatic reduction in food availability in grazing areas. About the 80% of parturitions in the goat take place during this season. Such conditions cause low kid survival rates and colostrum yields.

The objectives of the present works were to investigate some components of maternal behavior of local goats and if an energetic supplementation with maize would improve the mother and young behaviors. The main results come from our studies can be classified in two aspects: firstly, the description of some behaviors displayed by these animals under confinement conditions and secondly, the impact of feeding with an energy supplementation with maize few days before parturition on the maternal behavior.

Some spontaneous behaviors displayed by the mother goats

In the early work of our center, we study if some characteristics of the mother-young behaviors that can be displayed by the does (hiders) during postpartum would differs to what is already known in the sheep (followers). Between the first objectives, we study whether after 2 h of free dam-goat kid interaction, dams were able to establishes the maternal selective behavior when were submitted to individually interact for 5 min in a 2 × 2 m pen with their own or with an alien goat kid. During the test, dams were directly observed and frequency low-pitched bleats, acceptations to the udder, udder rejections,

aggressive behaviors and the time spent near the udder were recorded. From this first work we observed the following results: mothers emitted more low-pitched bleats (23.0 ± 7.0 *vs* 5.0 ± 1.0, P < 0.01) and accepted more times to the udder (2.0 ± 0.5 *vs* 0.6 ± 0.3, P < 0.05) their own kid than an alien one. Also, mothers display more aggressive behaviors toward the alien than to their own kids (4.3 ± 2.2 *vs* 0.1 ± 0.1 times, P < 0.05). However, udder rejections did not differ towards the alien or their own kids (2.4 ± 0.6 *vs* 1.4 ± 0.9 times, P > 0.05). Finally, the time spent near the udder tended to differ between their own and the alien kid (110 ± 30.0 *vs* 52.0 30.0 s, P = 0.07). From this first study we conclude that mother goats maintained under extensive. grazing conditions display maternal selective behavior as fast as within 2 h post-partum as has been previously reported in mother ewes.

Since the previous literature suggested that due the goat kids display a hiding phase after first four days of life, then it is possible that suckling behavior of their dams would increases towards the first week after birth when hiding phase of the goat kids ends. To verify this, we observed suckling behavior of twelve multiparous dams that had given birth to twin kids maintained under confinement conditions (García y González et al., 2017). Suckling behavior was recorded continuously during the first 6 h after parturition and in addition, this behavior was recorded from 0700 to 1300 h on days 1, 2, 3, 4, 5, 6, and 7 postpartum. From this second study we observed that intense suckling behavior of domestic goats under conditions of confinement is not significantly affected by the hider phase. Instead, nursing activity is reduced from the first to second day after parturition and over the course of the first week (Figure 1).

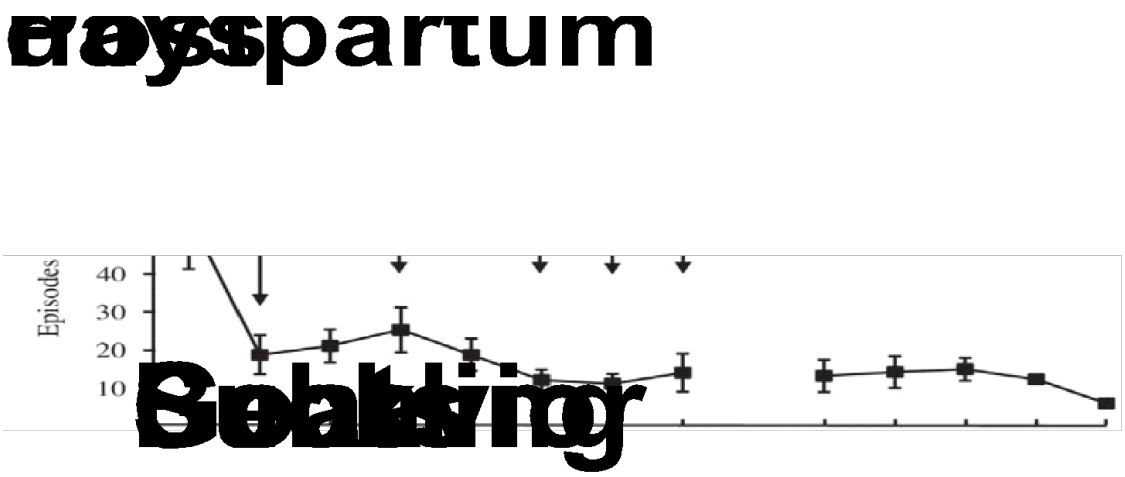

Figure 1. Spontaneous suckling frequency (mean ± SEM) recorded on a group of twelve local mother goats during partum and during first week after parturition. Asterisks and different letters denote significant differences (P < 0.01; Adapted from García y González et al. (2017).

As a first conclusion, we suggested that in spite of goat kid possess during postpartum a spatial relationship hider type no important differences are observed in terms of some components of mother-young relationships and bonding with previously reported in the sheep.

Influence of an energetic supplementation with maize during late gestation on goat's maternal behavior

From a series of studies carried out later, we investigated whether in grazing goats, an energy supplementation with maize during late gestation could improve some characteristics of the mother-young behavior. To test this, we used an experimental design which groups of pregnant goats were maintained solely with natural semi-arid grazing conditions (control goats) and other groups also maintained under the same grazing scheme, but in addition during the last 12 days of gestation they received 0.6 kg of flaked maize before parturition (maize goats). Maize was offered in the morning before grazing using small buckets. Provided maize substituted a part of the grazing diet providing 87.3 g of CP/kg DM and 3.06 Mcal of ME/kg DM. In all goats, mother-young behaviors were observed during the first 60 min after parturition. The results are show in the Figure 2., which indicate that maize supplementation improves maternal behavior. Thus, invitations to suckling were higher in the maize group than in the control group during the first 30 min after birth ($P < 0.05$). Duration of invitations to suckling tended to be higher in the maize group than in the control group ($P = 0.07$).

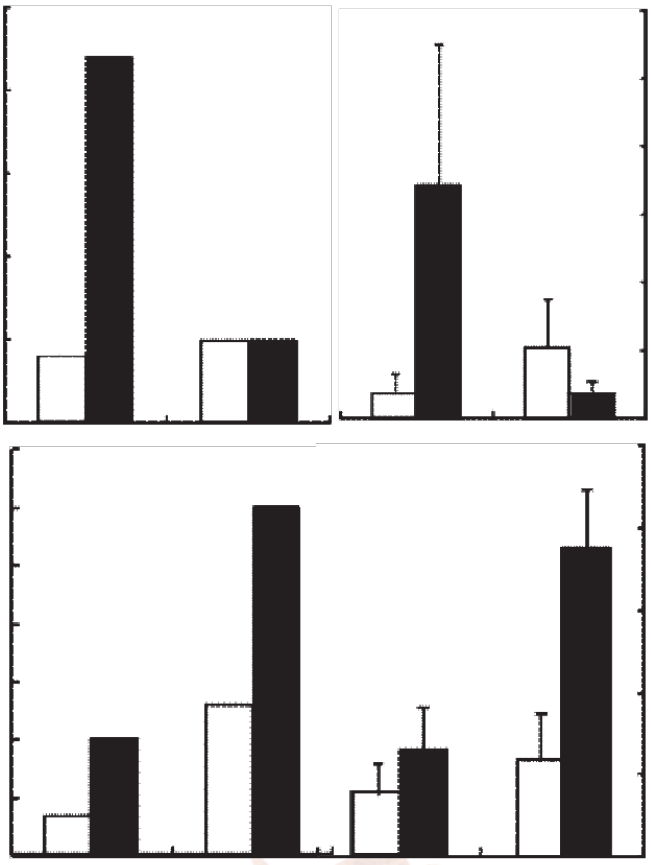

Figure 2. Total frequency and mean duration (±SEM) for invitations to suckling and suckling frequency in 2 consecutive 30-min periods after birth, by mothers maintained either under extensive grazing management without (□, control, n = 13) or with daily 0.6 kg of flaked supplemental maize/doe during

the last 12 ± 1.0 days of pregnancy (■, maize, n = 10). Differences between groups (**P <0.01). Adapted from Ramírez-Vera et al. (2012).

In this same groups of goats, we tested the hypothesis that in grazing goats the energetic supplementation with maize can improve the ability of the mothers to accept only their own goat kids and actively rejects an alien one in a 5-min test performed at 3 h postpartum (maternal selectivity). Dams were submitted to individually interact for 5 min in a 2 × 2 m pen with their own or with an alien goat kid (Ramírez-Vera et al., 2012). The number of udder acceptations towards their own kids was higher in the maize group than in the control group ($P < 0.02$; Figure 2) whereas the number of udder acceptations towards alien kids tended to be higher in the control group than in the maize group ($P = 0.06$). The frequency of udder rejections towards their own goat kid was higher in control mothers than in maize mothers.

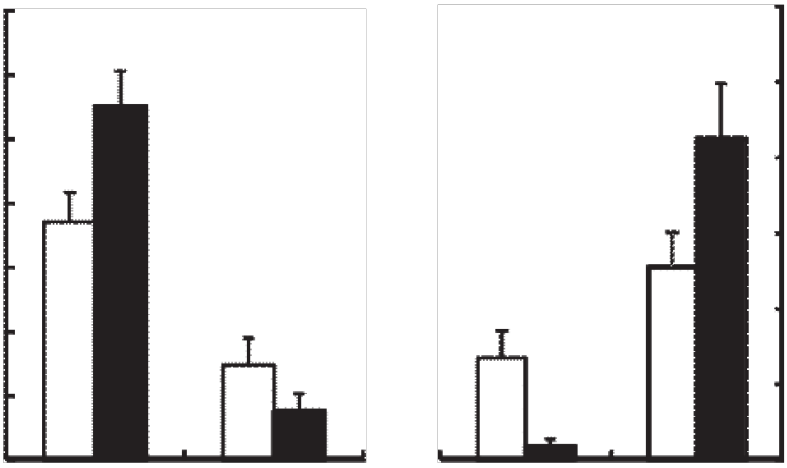

Figure 3. Mean number (±SEM) of udder acceptations or rejections display by mothers towards their own kid or toward an alien one. Goats were maintained either under extensive grazing management without (□, control, n = 37) or with daily 0.6 kg of flaked supplemental maize/doe during the last 12 ± 1.0 days of pregnancy (■, maize, n = 44). Capital and lowercase letters indicate a significant difference of behavior towards the own and alien kids ($P < 0.05$) between the same group. Differences between groups are indicated by asterisks ($P < 0.05$).Adapted from Ramírez-Vera et al. (2012).

With these previous results, we suggested a second conclusion that in goats maintained under semi-arid grazing conditions an energetic supplementation with maize few days before parturition results in an improvement of spontaneous maternal behavior and in a reinforcement of the maternal selectivity behavior of the mother at parturition.

Influence of an energetic supplementation during late gestation on the recognition of the goat kid by its mother

Using the same experimental groups above described (control and maize groups), we tested the hypothesis that an energetic supplementation during last 12 days of pregnancy in goats maintained under semi-arid grazing conditions would improve their ability to recognize their young at four hours after parturition. To this, in all dams, a two-

choice test (5 min) of maternal preference for their own goat kid from an alien one, in the absence of olfactory and visual cues was performed after 4 hours of continuous mother-young interaction. In all cases, it was ensured that every kid had made adequate suckling responses before separation. The test was performed on a triangular pen of 10 m per side; which consisted of 4 areas; Figure 4): waiting pen for the dam, neutral zone, and the contact area with the own kid and another for the alien kid. The base of the pen consisted of two boxes of 1 × 1 m where the kids were placed; these boxes were made of solid closed panels (Figure 4). During the test we recorded: Frequency of visits to the election zone of each kid (number of times that the dam visit the election zone of every kid, during the test), b).- Time spend near each kid (total time that spend the dam in the election zone of every kid), c).- Time spent gazing to each kid (total time that mother direct its head in the direction of the pen of every kid, regardless of the position of the goat in the testing pen).

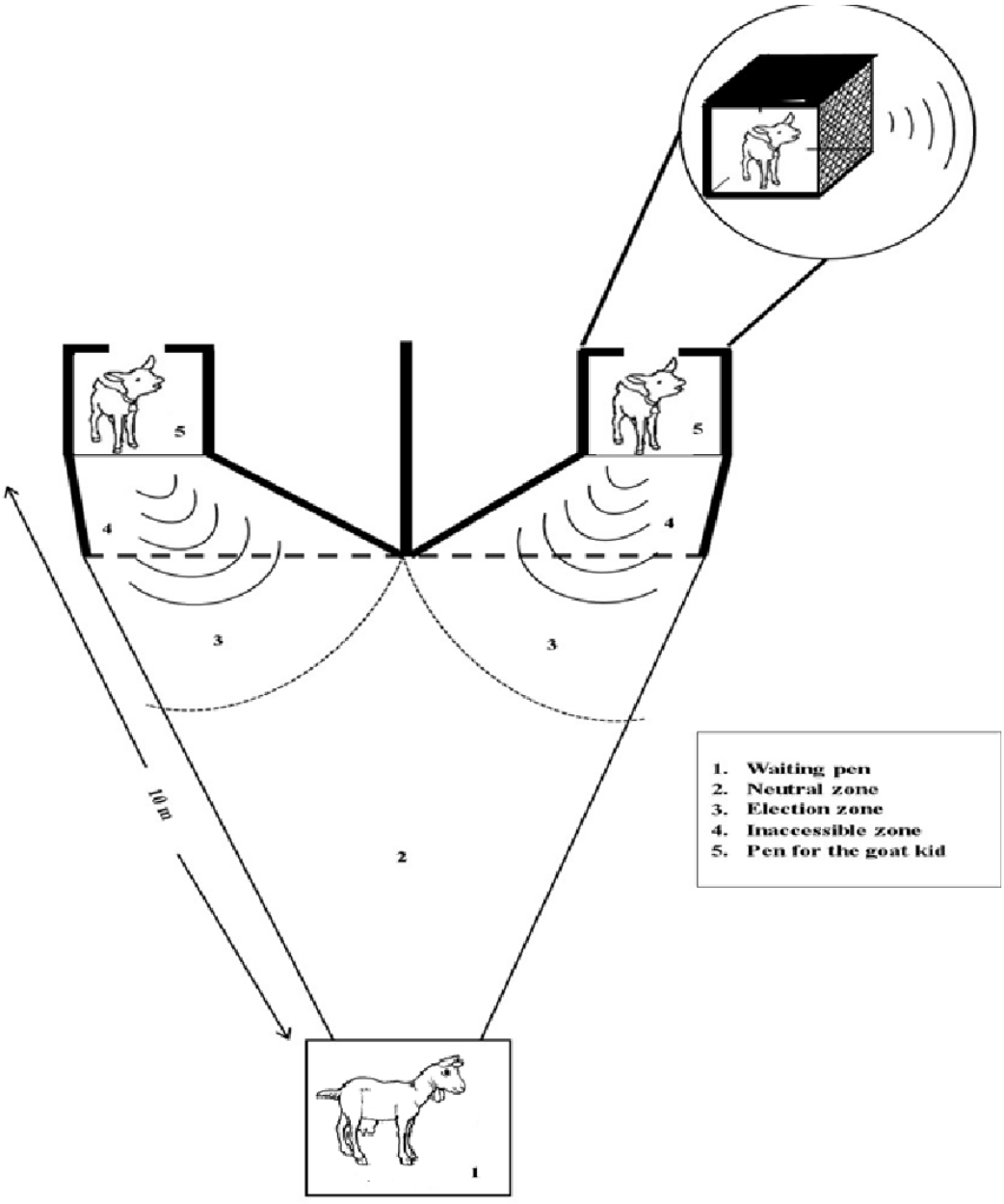

Figure 4. Testing pen used to test the distal recognition of their own kid from an alien one at 4 h post-partum based on live vocalizations. Bold lines indicate the presence of closed solid panels that helped lead live vocalizations of the kids towards the zone where mother goats performed a choice (own or alien). In the dialog box, the different areas in the pen test are indicated.

The results of this experiment are show in Figure 5. Thus, control mothers visit more times to the alien kid than maize ones ($P < 0.05$). The time that goats were near to the own kid was higher in maize goats than in controls ($P< 0.05$). On the contrary, the time near to the alien kid was higher in control goats than in maize ones. The same result was obtained in the variable the time gazing towards a kid.

The results of our study show that the domestic goats (*Capra hircus*), considered to possess a spatial mother-young relationship of type hider, a short-term energy supplementation few days before parturition facilitates the distal early discrimination of the new born kids by its mother.

General conclusions

The results obtained in our center show that in goats, whose offspring possess a "hiding" type of spatial relationship with their mother, the selective bond is established very early from 2 hours postpartum as reported in the sheep. Furthermore, these results show that the frequency of suckling behavior decreases from the first to the seventh day postpartum. Our results also confirms that in grazing goats, an energetic supplementation with maize improves maternal behavior and strengthens the mother-young bond.

Acknowledgments

The authors thanks to PhD Francisco Galindo for the English revision of this text.

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Socio-economic aspects of ruminant production

Practical Comprehension of the BVD virus for Disease Control and Virus Circulation

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Lattech Consultores SDS.

Objective:

This presentation focuses on understanding why BVDV (Bovine Viral Diarrhea Virus) control is complicated. Despite being described in the 1940s and the availability of vaccinations, herds still face problems with this virus.

BVDV has been extensively studied for decades, contributing to our understanding of its biology. It is classified as a pestivirus with a high mutation capacity and genetic diversity, resulting in different biotypes and genetic types. The virus possesses structural and non-structural proteins that can change, allowing it to evade the immune system and infect cells such as macrophages, thereby reducing the immune system's ability to control other diseases.

The significant costs associated with BVDV are primarily related to reproductive problems. The virus can persist within herds by producing persistently infected (PI) animals as a result of infection of a dam with non-cytopathic virus during early pregnancy (40-120 days). Approximately 1-3% of animals in the herd can be PI, but they serve as the principal source of future infections.

Vaccination has been employed for many years; however, the results have shown incomplete effectiveness. Both modified live vaccines (MLV) and inactivated vaccines have been used to aid disease control.

Effective diagnostics are crucial for controlling this virus. Identifying PI animals allows for their culling, thereby eliminating a significant source of future infections. However, the use of certain vaccines can complicate the identification process, highlighting the need for additional diagnostic tools.

Sustainability and environmental impact

Cattle Health, Husbandry and Genetics: the Sustainability Connection

MSc Ruaraidh Petre

Abstract

The global food system faces increasing challenges, needing to reduce the impact of the current system while feeding a growing population. Livestock production is part of this, and animal health, breeding and genetics can contribute significantly to livestock sustainability. The Food and Agriculture Organization of the United Nations (FAO)'s (2023) report gives guidance on possible improvements in the livestock supply chain, while increasing food accessibility. Key takeaways include productivity increases (per animal) and breeding (reducing calving interval and using better genetics) which are the major contributors to reducing the overall footprint. Adoption of cattle vaccinations against common diseases can reduce the footprint and the size of the supporting herd. We urgently need investment in all aspects of animal health and welfare to help drive the combined benefits to human lives, health and livelihoods that better animal production can deliver.

Introduction

Animal Health and Animal Welfare are part of the definition of Sustainable beef. Without caring for cattle properly, we cannot claim that we are operating sustainably. This is an ethical imperative and one that bears emphasising from the beginning. We will look at the ways in which health and welfare reinforce each other, and contribute to a number of aspects of sustainability. We will also look at how actions that may be primarily targeted at sustainability can reinforce health and welfare.

First, however, remember it is the ethical imperative that there is no sustainability without animal health and welfare. We have a duty of care to the animals we raise for food, and everyone involved in the process needs to be doing what they can to give them a life worth living.

Sustainability

The global food system faces increasing challenges, needing to reduce the impact of the current system while feeding a growing population. At the same time, food production itself is affected by climate change. Uniquely amongst industries, food production has the ability to contribute to both adaptation and mitigation of climate change. Livestock are very much par

As time goes on, we will find increasingly that areas that were capable of producing crops becoming unable to do so, due to climate change. As it is, the majority of land we have available for agriculture cannot grow human edible crops. Ruminants can upcycle roughage that we can't eat into highly nutrient dense meat.

All industries need to be working on reducing emissions, and the livestock industry has been on a decreasing emissions trajectory per pound of beef for decades, continuing into the present and beyond. These efforts are in both High-Income Countries, and in

countries such as Brazil. There are numerous ways we can reduce emissions associated with beef production, in intensity, and if adopted widely enough, in absolute terms. Despite widespread public commentary, the details of how beef supply chains can reduce emissions are still not well understood by those outside the industry.

The Global Roundtable for Sustainable Beef (GRSB) and our members have set an ambitious goal for GHG emissions: A reduction of 30% by 2030, on a pathway to net zero. The short-lived nature of methane as a GHG means that emissions do not have to cease entirely to stop warming. In fact any reduction in emissions from a given herd means that the herd is contributing to reduced temperature forcing.

It will not be easy to accomplish this on a global scale, primarily because increases in demand in developing countries stimulate increased supply through larger herds. We need to accelerate investment in those regions of the globe to ensure that the increasing demand from larger human populations is met in sustainable ways, by producing more efficiently rather than expanding the herd. FAO's report (FAO 2023) released during COP28 outlines how increased global demand for meat can be met without increasing emissions but decreasing them. GRSB set our goal to focus minds and investment to rise to this challenge, and we are bringing participants in the supply chain together to identify and address common barriers. Goals to reduce GHG emissions have also been set in many parts of the food system. Providing transparency and insights on good husbandry, health and welfare along the chain also contributes to greater value for both primary producers and buyers (Maia de Souza *et al*. 2017).

This brings us to how Sustainability is defined. The answer to this question is exactly what a group of farmers and ranchers, processors, input providers, retailers and NGOs wanted when we formed the Global Roundtable in 2012.

It took GRSB 2 years to finalise our definition, which was agreed upon by our members and launched at the Global Conference on Sustainable Beef in Sao Paulo in 2014. It encompasses 5 principles covering Natural Resources, Animal Health and Welfare, People and the Community, Food, and Efficiency and Innovation. It also includes 36 criteria, and the document is 11 pages long. That might seem a bit unwieldy, but it's important to understand your priorities before embarking on a journey, particularly one as large as transforming a significant industry feeding billions of people.

GRSB defines sustainable beef as a socially responsible, environmentally sound, and economically viable product that prioritizes Planet, People, Animals and Progress.

When asked to come up with a brief definition of my own for "sustainable production" I use "Production that meets the needs of today's population without compromising our ability to meet future needs," which aligns with GRSBs and with terms such as regenerative or agroecological.

In 2021, GRSB launched three global goals covering Climate, Nature Positive Production and Animal Health and Welfare. This year we will launch our 4th goal on Social Impact.

These days, it is hard to open a newspaper or a newsfeed without reading an article about how modern agriculture impacts the planet, mostly in the negative. First among the culprits identified is usually beef production, and the main complaint is regarding beef's enteric methane emissions, followed by deforestation / land conversion. We have to acknowledge those issues, and like every other industry, we do have to work to

minimise the negative impacts of our production on nature and climate. Having said that, we also need to acknowledge that people need to eat and that 2/3 of our agricultural land cannot produce human edible crops.

Ruminants upcycle grass and other roughage into nutrient dense meat with highly bioavailable minerals and vitamins that are deficient in significant portions of the human population. The current narrative is damaging because it offers no solutions, while pitting the consumer against the farmer. Many of the problems we see in modern agriculture actually do not stem from farmers, but from policy and other signals they receive from governments and companies further up the supply chain. The consumer pays less for food now than they did in the 1970s (in constant pricing), and it is generally the farmer that has lost most of that income.

So, while avoiding "Carbon Tunnel Vision", what does animal health and welfare contribute to reducing the impact of beef?

This is not purely a question of efficiency, but efficiency is a very significant contributor to it. Consider the dairy cow that can supply milk and a calf that can be finished for beef every year. Contrast that cow with one that has insufficient feed and health care, to the extent that she only produces a calf every 4 years. The first dairy cow has a much lower footprint per kilo of product than the second. In emissions per year, the first dairy cow is probably higher, but the other cow has produced nothing.

Now consider an expanding herd: Without adequate inputs, feed, healthcare and good husbandry we see a huge impact, due to the short-lived nature of methane. Contrast that with a herd that has increased production per head over three decades while *decreasing* in size. The latter has actually contributed to lowering climate impact, while feeding more people.

Resilience

The concept of resilience is defined as "a system property that, in its most desirable form, allows thriving systems to cope with change. The systems perspective blends ideas from complexity theory, ecology, and social sciences to understand dynamics of change and alternate regimes in social-ecological systems." (Hodbod et al 2022). As it happens, measures designed to increase resilience and adapt to climate change may also favourably benefit biodiversity in agro-ecosystems, as well as assisting in mitigating climate change: "They're not really about carbon farming, even though that's an outcome... They have a focus on rebuilding resilience into the landscape and with that comes productivity." (Gosnell et al. 2020).

The framing of sustainability in terms of resilience gained considerable ground as a result of the Covid-19 pandemic which coincided with the widespread epidemics of African Swine Fever (ASF). What both diseases demonstrated was that shocks at this scale have a very disruptive effect on supply chains with negative impacts on consumers and producers. While value chains can be efficient when there are no disruptions, 2020 showed they are poorly adapted to cope with such large human or animal disease outbreaks (Hodbod *et al* 2022), underlining the keystone importance of animal health and zoonoses in sustainability.

Animal Health and One Health

Following on from the concept of resilience as outlined above, the interrelation between animal and human health is critical. The example of twin shocks to the food system of Covid-19 and ASF underlines how both zoonoses and livestock disease can impact human lives and livelihoods.

I have had the good fortune to work with livestock and people in a wide range of different environments. In each of those contexts, animal health was a major focus for me and the people I worked with.

In my early career I worked on and managed sheep and beef farms, where vaccinations, anthelmintics and acaracides all played their part in ensuring those farms were productive and profitable. Given the very low returns in farming in general, and livestock in particular, these routine treatments made the difference between a viable farming system and one that could not pay for itself.

In Scotland, we vaccinated against a range of clostridial diseases and BVD, and regularly rotated our anthelmintic treatments to try to avoid the development of resistance. At that time, there were cases of BSE in the country, so we had to be particularly careful where we sourced our cattle. As we were in the west of the country, in a very wet climate, we also had persistent issues with foot rot in sheep that required a high degree of vigilance and development of a pasture rotation to help minimise the problem. Mineral supplementation was important in cattle and sheep, with both copper and magnesium required on those pastures.

In Australia, I worked on some very extensive properties in the Northern Territory. On one, we had to vaccinate against botulism, and those were still the days of regular Brucellosis and Tuberculosis testing. One of the routine jobs, next to mustering, branding and drafting, was the TB intradermal caudal fold test, with all of the reactors destroyed. That government program ultimately led to the eradication of TB from Australian herds (Glanville, 2023).

I also worked on a program that included rinderpest vaccination in Southern Sudan as part of the global rinderpest eradication effort (Catley, Leyland & Bishop 2005). Following that. I ran a veterinary program in Afghanistan (Schreuder, 2020) which included paravet training, and the import and distribution of veterinary vaccines and medicines covering the whole country. As Afghanistan still has endemic anthrax, and both Peste des Petits Ruminants (PPR) and Crimean Congo Haemorrhagic Fever (CCHF) were present in the country, the importance of having a good network of paravets and a reporting system under relatively primitive conditions cannot be overestimated.

In each of these contexts there was never any doubt about the importance of animal health to the livelihoods of the people involved, and the sustainability of the system. The paravet model in Afghanistan emphasised the importance to those communities. Their level of understanding of herd immunity was demonstrated by villages paying for vaccination of all animals, ensuring that all were covered and paid for, even when individual livestock keepers could not afford vaccine.

Yet there are large areas of the world to this day where availability of vaccines and treatments is limited, where the distance livestock keepers travel makes it unrealistic that they will keep up regular vaccinations or treatments required. Simple problems like copper deficiency in sheep resulting in swayback in lambs remain unrecognised or

untreated simply due to a lack of available qualified people on hand to recommend supplementation. In Africa and Asia, with over two thirds of the large ruminants in the world, they produce less than one third of the products from them. We urgently need more investment in veterinary medicine in lower income countries to improve the sustainability of animal production and the availability of nutritious animal source foods for the growing population.

If you have not read the Oxford Analytica's Health for Animals report that came out last year (Oxford Analytica, 2023) I thoroughly recommend it. I will give you some examples from it that elaborate on the impact of health on sustainability:

> "A 60% global vaccination rate for beef cattle correlates to a productivity rise of 52.5%. This rise is equivalent to the beef consumption needs of 3.1 billion people."
>
> "Globally, the modelling found that a 40% global vaccination rate for cattle in a given year is associated with a 5.2% reduction in land required for production."
>
> "Every 1% reduction in beef cattle disease rates would: a) increase production enough to meet the average beef consumption needs of 317 million people b) increase producer revenue by US$3.2 billion."

I used the heading One Health and we should not underestimate the interaction between animal health and human health. It is not just through zoonoses but also through the impact of good stewardship of pharmaceuticals that ensures they remain effective for both people and animals. Antimicrobials are the products where most concern is expressed about use in animals, and livestock keepers and their vets need to make certain they minimize the use of critically important antibiotics for human health. Needless to say, doctors treating people should be similarly cautious in their use of critically important antimicrobials.

In beef cattle, the products of most concern are macrolides due to their importance in human medicine and their use in Bovine Respiratory Disease. This emphasises the need to condition cattle, vaccinate them and get them as resistant to BRD as they can be to minimise the need to use macrolides both in pastured cattle and in feedyards. (BRD is the most common reason for antibiotic treatment before weaning).

Animal Welfare and Husbandry

Everyone instinctively understands how good animal health contributes to animal welfare. We have all been sick at some stage and recognise that when sick, we are not feeling good. It fair to assume the same situation exists for animals. Not surprisingly, this is confirmed through animal behaviour, appetence, cortisol levels etc. No surprises there. What about the other way around? Does improved welfare contribute to better animal health?

To a room of veterinarians, the answer should not surprise you: Better welfare certainly leads to better health outcomes. There are some concrete examples of how that works in practice, Painful procedures like disbudding, when done early with anaesthesia and analgesia, lead to calves that eat better and are less likely to succumb to infections, as compared to those without. Similarly, fenceline weaning is recognised as being less stressful on calves leading to lower levels of movement and increased appetence. This contributes to producing calves better conditioned and resistant to disease prior to later

transport for backgrounding or finishing. Apart from the ethical reasons for good welfare stemming from good husbandry, it makes good financial sense for all those in the chain to ensure that cattle are well treated from birth onward.

Other issues may be considered good husbandry rather than welfare *per se,* such as ensuring micronutrients are sufficient through mineral supplementation, avoiding excessive poaching in fields, excessive manure and overcrowding in housed or penned cattle, all clearly contribute to welfare and directly to health. It has been demonstrated that reduced space per animal in outdoor feedlots impoverishes animal welfare (Macitelli *et al.,* 2020;

Vaccinations cannot overcome poor husbandry or poor welfare. Treating stock that are sick as a result of poor husbandry or welfare is a double and avoidable expense. Good health, feed and husbandry all contribute to reducing calving interval, a key efficiency parameter. This, in turn can reduce the size of the supporting herd and pressure on natural resources.

Genetics

Breeding and genetics can provide cattle suited to a wide range of environments. Breeds with adaptations to different climates, through e.g. *bos indicus* genes or short haired coat, or disease resistance to tryponsomosis, through African taurus cattle such as the N'dama, already exist. Clearly adaptations to where they live in terms of climate, available forage or topography and endemic diseases are the foundation of having a productive herd.

Cross breeding resulting in hybrid vigour has been used for generations to increase the output of cattle, though such cattle still have to cope with their environment. The benefit of cross breeding can be tempered by the level of adaptation of the two breeds concerned and the inputs livestock keepers are able to provide them.

More recently we have seen the introduction of genes into cattle through CRISPR gene editing. This can deliver a whole new trait without the need for cross breeding of two otherwise rather different breeds, and can thus accelerate progress in one direction. In 2022, the FDA in the USA declared cattle that had the slick coat gene edited to be safe to market. This holds a lot of promise for adaptations required to cope with a changing climate, as well as disease resistance genes that could be transferred to animals with other desirable traits including productivity, polled genes and others.

Within breeds, and indeed within individual herds, there is considerable variation in genetics that are related to sustainability. Residual Feed Intake is one of these, and biomarkers associated with RFI are now included in some EBVs and EPDs. Since feed represents around 70% of costs, this can represent a significant savings to producers in terms of feed required to achieve a given output.

Similarly there are now bulls with a low methane trait, which could reduce the methane emissions in the (initially dairy, later beef) herd by as much as 1.5% per year, with widespread adoption. The variability in methane emissions within and between herds of the same breed is considerable (CV ranging from 22% to 67% within farms, from a sample of commercial dairy herds (Bell, M.J. *et al* 2014)), there is considerable scope for selecting for low enteric methane, where the technology is available to measure this.

Individual producers make selections based on their system and priorities. Physical environment is not the only purpose of breed selection. Clearly, those using cattle for multiple purposes will have a different set of selection criteria from those who are seeking to maximise marbling or average daily gain. Preservation of genetic diversity in cattle is important as it may help us meet challenges in the future. Genomic tools that enable us to identify sustainability and production related traits can also be used to monitor and conserve genetic diversity (Eusebi *et al,* 2020).

Fortunately, the rise in availability of data on EBVs and EPDs can help producers target their selection to work in their environment and their system. Availability and adoption of AI services and genomic data is still patchy, and thus the potential for genetic advancement is far from being met in the beef industry, compared with dairy or the swine industry.

The use of beef genetics on dairy cows is making a substantial difference to the efficiency of beef production in the US now (Poock *et al*, 2021). 66% of cattle slaughtered in Europe come from the dairy herd, and the figure for New Zealand is around 40%. There is opportunity for significant savings in terms of the supporting herd through use of beef on dairy.

The genetics of the microbiome should also be considered when looking at efficiency traits. Microbiome–host interactions play an important role in efficient nutrient digestion and cattle health. Microbial markers and gene expression in the rumen microbiome are now being used to identify efficient cattle (Terry *et al* 2021).

Contributions towards lowering the hoofprint of livestock

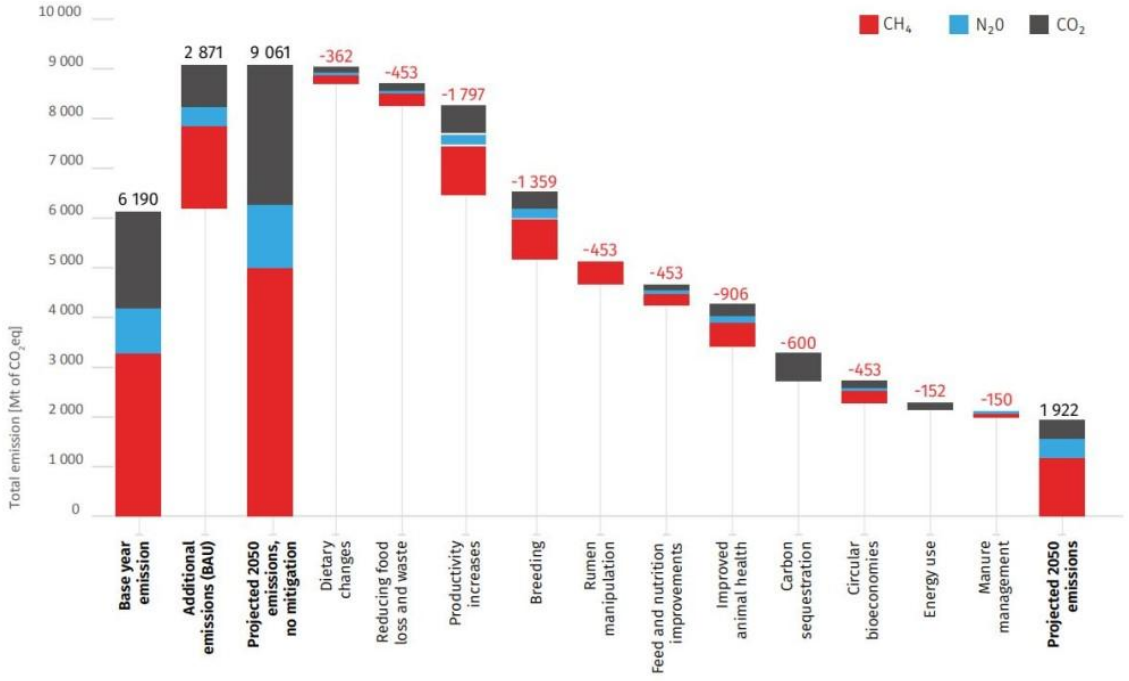

Fig 1. Base year and projected emissions from livestock systems shown as a waterfall chart with a range of mitigation measures applied to 2050 with their technical potential (FAO, 2023)

FAO released a constructive report (FAO, 2023) regarding livestock and climate impact at COP28 In it, they reported we need more meat and animal sourced food, and in order to meet demand without increasing the climate burden, there are a number of things we can do. Animal health and welfare are very much part of that picture.

The FAO report gives us some confidence that emissions reductions are possible in the beef supply chain, while increasing food accessibility. Key takeaways from that report (see fig 1) are that productivity increases (per animal), and breeding (reducing calving interval and using better genetics), are the major contributors to reducing the overall footprint. Animal health, feed quality and reducing food waste are crucial components, and on the other side of the equation, increasing soil carbon sequestration can also play a part. On top of those more classic approaches, there is some promise in terms of rumen manipulation, feed additives or vaccines to reduce production of enteric methane at its source. In some intensive systems, manure management and biogas capture can turn emissions into an energy source.

There is science to back up these FAO and Health for Animals figures. A sustainable future for *all* means investing to ensure that developing countries progress on a more sustainable path than High Income Countries have over the last 2 centuries.

It bears repeating: Animals experiencing good health and welfare are more productive than those that don't. It translates into more product per animal or per day of life, and therefore lower impact. In terms of climate impact, clearly that means lower emissions per kg. This is how productivity increases represent the largest contributor to FAOs pathway to lower emissions.

Beyond that, better health and welfare has resulted in a *smaller supporting herd* for the amount of beef, milk and leather produced. We have seen this development in many countries over the past several decades, which in turn means a smaller footprint and at least the *opportunity* for more nature positive production.

US total cattle inventory peaked around 1975 at 132 million head, and is today 94 million, a reduction of 29%, but *beef* production is 17% higher at around 12.2 million tonnes in 2022. If the US still produced in the same way as in 1975, the country would need a herd of 60 million more head than it actually has, using more water, land and feed. 60 million head of cattle represents a similar number to the total number of wild bison in North America prior to the arrival of Europeans.

All countries can make that transition and many, for example, in Latin America are following that trajectory. Brazil has intensified over the past decades, but has continued herd expansion, meaning that there are a larger number of cattle on a similar area. The total herd size in Brazil is currently around 220 million head producing around 10 million tonnes of beef in 2022. There is still considerable room for land sparing by improving productivity per animal. The connection between the expansion of the Brazilian cattle herd and deforestation is often cited, and it is certainly true that cattle are invariably present at the deforestation frontier. That is not to say that all deforestation can be attributed to beef production.

There are many factors that contribute to deforestation including timber extraction, land value speculation (the value of newly cleared land is immediately 3-5 times more than land with forest, and once it is ready for crop cultivation, that land can be worth as

much as 20 times as much as forested land), and the desire to bring more land into crop production. Crops cannot be cultivated in newly felled land, so cattle are used as an intermediate stage to establish occupancy and some return until infrastructure and land condition permit cropping. This complex of drivers includes cattle, but ultimately is about economic development. In order to cease deforestation in such countries, the world as a whole needs to value forested land higher than it currently does. The total footprint of the cattle herd in Brazil has not grown since the 1990s, but it has moved with the frontier. With improved productivity, the footprint of the herd could shrink, as has been the case in the US.

While deforestation receives a large amount of attention, particularly in the popular media, grassland ecosystems are the most threatened on the planet. In many cases they are capable of producing human edible crops and are vulnerable to conversion.

Regenerative approaches to land management are an important part of reducing current and future impacts of livestock production and can contribute to ecosystem services for the water cycle, biodiversity and soil carbon sequestration. Such management has potential to reverse land degradation (Angerer *et al* 2023, Sandhage-Hofman, 2023). There are more than 1 billion ha of degraded rangeland around the world, and further large areas of degraded croplands, we need investments to encourage these management approaches. Restoration could also relieve pressure on conversion of forests and other ecosystems.

Well managed grazing can preserve and enhance the biodiversity and ecosystem functions of native grasslands, regardless of the species involved, just as a lack of management can result in degradation even where wild species are involved (Beschta *et al,* 2020). The fact that regenerative systems contribute to outcomes that help adaptation to and mitigation of climate change as well as enhancing biodiversity, leads to the conclusion that they can also contribute to economic and social dimensions of sustainability, even if they are not necessarily maximizing yield per unit area (Rowntree et al. 2020).

Within such approaches, silvopastoral systems (SPSs) (Mauricio R.M *et al.,* 2019) offer a triple win in (mainly) tropical environments. While SPSs can enhance biodiversity, carbon capture and water cycle, they can simultaneously increase carrying capacity, income and resilience of the production system. They contribute to improved animal health and welfare through reducing surface temperature and increasing shade, as well as providing a wider diversity and higher quality of feed and micronutrients. It seems clear that this is an area in which considerable investment should be directed. The establishment costs of SPSs is generally higher than pure grazing systems, but the return on investment is only a few years and the benefits to people, planet and animals are clear.

Conclusion

To conclude, it is clear that transitioning livestock production to more sustainable models is possible, but not automatic. Current system dynamics lack resilience in the face of multiple challenges including biodiversity and climate crises. Trade arrangements that favour a race to the bottom and short term political decision making are all obstructions to transformation. We need investment in all aspects of animal health and

welfare to help drive the combined benefits to human lives, health and livelihoods that better animal production can deliver.

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Short communications

Novel approaches for helminth control in ruminants: special emphasis on gastrointestinal nematodes and *Dictyocaulus viviparus*

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The livestock industry faces a range of challenges arising from consumers' awareness towards sustainable farming. Prominent concerns include environmental issues such as greenhouse gas emissions and climate change (Windsor, 2021; Zafrilla, 2020; Torres, 2020), animal welfare, and the need for improved productivity and market access (Eeswaran, 2022; Kumar, 2021). The industry also grapples with the complexities of demand-side challenges, including evolving consumer preferences and market segmentation (Moran, 2021). To address these challenges, a multifaceted approach is required, encompassing technological innovations, improved animal health and welfare, and the integration of environmental considerations into production practices (Kumar, 2022).

Understanding consumer preferences in the context of livestock products is essential for shaping production practices, fostering sustainability, and meeting evolving market demands. Consumer preferences are influenced by a myriad of factors, with food safety and quality being the main attributes in shaping consumer trust and loyalty towards livestock products (Grunert and Wills, 2007). Animal welfare considerations play a pivotal role in shaping consumer choices, emphasizing the increasing importance of humane treatment of animals in livestock production (Hemsworth *et al*., 2015). Ethical and sustainable practices are gaining prominence, reflecting a shift in consumer values towards environmentally responsible and socially conscious agriculture (Vanhonacker *et al*., 2010). Furthermore, as consumers become more conscious of health and nutritional aspects, there is a growing demand for transparent information regarding production methods (Verbeke *et al*., 2010), thus, the role of labeling and certification schemes, is crucial in conveying information to consumers about the production process, allowing them to make informed choices aligned with their preferences (Haghiri *et al*., 2021).

Succinctly, the livestock industry must be aware of emerging trends such as plant-based alternatives and lab-grown meat that are reshaping the landscape of consumer preferences, leaving the livestock farming under latent risk of losing sustainability in the medium-long term (Mendoza-de Gives *et al*., 2023). Therefore, alternatives to address animal health, welfare, and performance meeting the new standards established by demand are needed to gain social acceptability and ecological balance, while having to remain economically viable.

Over the past decade, considerable efforts have been done to offer management alternatives focused on addressing the problem. Among the most publicized

alternatives to mitigate the impact of traditional livestock farming are organic farming, silvopastoral and dual-purpose production systems. According to reports made by the European Commission for Agriculture and Rural Development (ARDEC) and the National Agricultural Statistics Service (NASS-USDA) during the last year (2023), the certification for organic production units has had an annual increase of 5.7%. Consumption preferences justify this increase, since compared to traditional production systems, organic production houses 30% more biodiversity, energy expenditure is reduced by 45% and emits 40% less carbon. However, these production systems come with risks for both animals and producers; with nutrition and parasitosis being the two most prevalent challenges. However, consumers not only expect animals to be raised under these ecological friendly systems but also expect for welfare and health to be achieved without the use of chemicals, including dewormers.

Internal parasitosis represents one of the top ten health problems within grazing livestock systems. Herd prevalence rates of up to 98% have been reported, affecting the health, welfare, and performance of ruminants (Fitzpatrick, 2013). Furthermore, recent research has identified challenges such as mastitis,
lameness and high parasite loads on organic livestock farming (Akerfeldt, 2020, Toro-Mujica, 2012). The prevalence of gastrointestinal nematodes (GIN) and lungworms in cattle often varies by region due to differences in climate and environmental conditions. Warmer and more humid climates are generally conducive of higher parasite burdens. However, parasites' high capacity for adaptation to climate change has modified the epidemiology and parasite ecology, becoming increasingly more common to find parasite species believed to be endemic to the tropics in temperate zones, and vice versa (Kaplan and Vidyashankar, 2012).

Helminths infecting cattle include a wide range of species that affect different systems and mostly cause destruction of glandular or epithelial tissue. Helminths can be classified into two large groups: nematodes (roundworms) and plathelminths (flatworms, cestodes and trematodes). Gastrointestinal nematodes (GIN) and *Dictyocaulus viviparus* are roundworms that affect the digestive (abomasum and intestines) and respiratory systems, respectively. The degree of infection will depend on various factors related to the parasite, the host, the environment, the type of production system and its management. However, it has been reported that even animals with low parasite burdens (<200 epg) present delays in the developmental milestones, such as: loss in daily weight gain, delay in growth, decrease of voluntary intake and in milk production, low fertility, increase in the incidence of mastitis and an increase of costs associated to treatment and management (Li and Gasbarre, 2009; Li *et al*., 2009; Stromberg *et al*., 2012). Studies have estimated the economic loss of GIN in Mexican cattle to rise to 445.10 billion US dollars per year (Rodríguez-Vivas *et al*., 2017). Furthermore, a decrease in daily milk production of 0.5 kg/cow/day and a reduction in the percentage of protein in milk of 0.02% has also been reported (Charlier *et al*., 2016). On the other hand, animals infected with *D. viviparus* have shown a reduction in milk production greater than 50%, mortality rates of 20-33% and average costs of 150-167 EU per cow (Liu et al., 2023). Regarding reproductive parameters, a marked divergence has been observed in the growth rates of up to 30 kg and in body condition scores of up to 1 point (scale 1-5)

between dewormed and non-dewormed heifers (Loyacano *et al*., 2002); indicating that dewormed females reach puberty at an earlier age, and thus, calving rates are affected (Zinsstag *et al*., 1997). Similarly, higher pregnancy rates and heavier weaned calves (> 20 kg) have been reported when heifers are dewormed at the beginning of reproductive management (Loyacano *et al*., 2002). In conclusion, both GIN and lung worms affect livestock health, welfare, and performance, thus, parasite control should be properly addressed specially under organic farming and grazing systems.

The control of helminth infections in ruminants is a complex and evolving challenge, with the routine use of chemotherapeutics being unsustainable (Sutherland, 2011). Since its discovery more than 60 years ago, the cornerstone of parasite control has been the chemoprophylaxis, due to its broad spectrum and positive impact on animal performance (Jackson and Miller, 2006). However, the emergence of resistant and multi
resistant strains of internal parasites, along with consumers' preferences concerning ecological impact, chemical residues, and animal welfare, has raised doubts about the short to medium-term sustainability of chemoprophylaxis. Vercruysse (2018) proposes a vision for helminth control in ruminants by 2030 which includes the integration of scientific advances in diagnostic tools, innovative control approaches, and the sustainable use of anthelmintics. To address the latter, alternative control methods are being explored, including targeted treatments and selective treatments (Charlier 2014). Targeted Selective Treatment (TST) has emerged as a strategic approach for managing nematode infections in cattle, offering a precise and sustainable alternative to broad-spectrum anthelmintic treatments, controlling parasite infections while reducing the selection pressure for anthelmintic resistance (Charlier, 2014, Hoste 2002). These methods aim to preserve anthelmintic efficacy while effectively controlling nematode-induced production impacts. Novel lines of research focused on parasite control alternatives have shown substantial progress over the last decade, and in recent years, the focus has been on establishing integrated and sustainable schemes of parasite control, which are highly needed. Thus, it's important to be aware of some of the most researched alternatives for parasite control (Jackson and Miller, 2006):

i) Control strategies for suprapopulations (grazing management and biological control). ii) Control strategies for infra-populations (pharmacological, immunological and use of bioactive plants).

I. CONTROL OF SUPRAPOPULATIONS. Refers to the control of the nematode stages found in the pasture (egg, L1, L2 and L3 of NGI and L3 of *D. viviparus*), which represents 80% of the parasite population within a Livestock Production System (LPS).

1.1. GRAZING MANAGEMENT. The main objective of grazing management is to provide animals with safer pastures (low infectivity), which can be accomplished considering three main objectives: i) reduce larval density, ii) exploit the natural mortality rate of infective larvae and iii) accelerate larval mortality (Torres-Acosta and Hoste, 2008). In general, all strategies aim for an epidemiological modulation through the implementation of one of the three management schemes:

A. **Silvopastoral systems.** Silvopastoral systems (SPS) emerge as a sustainable and multifaceted strategy for controlling GIN infections. Silvopastoral systems offer a holistic and sustainable alternative, utilizing the synergies between trees, forages, and livestock to dilute the infection through the selection of the ruminants' diet, thus, minimizing GIN impact upon animal health, welfare, and performance. Furthermore, studies report that the implementation of SPS favors the biological diversity of soil fauna, such as dung beetles, which can reduce the populations of GIN and *D. viviparus* free
living stages due to a rapid decomposition of organic matter and the destruction of eggs and/or larvae during their breeding process (Soca *et al*., 2007). On the other hand, the incorporation of legumes in SPS can also contribute to parasite control through both immunonutrition and the AH-like activity of plant secondary metabolites (PSM) (von Son-de Fernex *et al*., 2018).

B. **Rotational grazing.** Rotational grazing has emerged as an effective and sustainable strategy for GIN control, leveraging pasture management to reduce parasite burdens by having an appropriate alternation between the period of use and rest of the paddock and the livestock stocking rate. It consists of dividing a pasture into several grazing sections, leaving areas inaccessible to animals, which, together with an adequate stocking rate, allows the life cycle of nematodes to be broken by starvation and/or by the effect of temperature and solar radiation (Torres-Acosta and Hoste, 2008). The peak of GIN and *D. viviparus* larval mortality occurs with rest periods of 32-40 days (Ploeger, 2011). Although this period may vary depending on the environmental conditions to which they are exposed, the rotation calendars could be adjusted to consider the peaks of larval mortality of both nematode species.

C. **Mixed/alternate grazing.** This system is based on two principles: i) combination of productive species that cannot be infected by the same parasite species (cattle/sheep; cattle/goats; cattle/equines; cattle/deer) and ii) combination of physiological stages (adult/calf) where the innate resistance of the adult dilutes the infectivity of the pasture for the offspring (Tapia-Escárate *et al*., 2021; Nielsen 1999)

1.2. BIOLOGICAL CONTROL. GrØnvold *et al*., (1996) defines biological control as *an ecological method designed by humans to reduce the population of parasites or pests to acceptable subclinical densities or to maintain those populations at non-harmful levels using live antagonists*. There are multiple agents that have been reported as biological control alternatives for nematodes control, among which we can find:

A. **Arthropods:** Together with their ecological service, Dung beetles (DB), have demonstrated in both *in vitro* and *in vivo* studies that some species could modulate the parasitic population in pastures. As part of their reproductive behavior, DB break down and bury cattle feces to form brood masses; which are buried at depths of up to two meters. This behavior reduces the infectivity of the pastures by reducing the availability of suitable habitats for GIN larvae development. Among the beetle species reported as parasitic control agents we can find: *Copris incertus* and *Digitonthophagus gazella*; species that demonstrated on *in vitro* trials their ability to reduce the GIN larval population

between 49% and 57% (von Son-de Fernex et al., unpublished data). While species of the genus *Aphodius* (*A. vittatus, A. coloradensis, A. fimetarius, A. homisus and Canthon sp. Aphodius vittatus and A. coloradensis*) managed to reduce the *Dictyocaulus hadweni* L1 population in feces by 84.7% (Bergstrom, 1983). Similarly, it has been reported that the presence of DB has the capacity to reduce the number of sporogonies of the *Pilobolus* fungus in feces between 56.9% and 80.9% (Beynon et al., 2015; Biggane and Gormally, 1994). *Pilobolus* spp., is a fungus with worldwide distribution that germinates in the excrement of ruminants, and is characterized for an explosive discharge of its sporangia facilitating *D. dispersion* in the paddocks. The L3 of *D. viviparus* exerts a vertical migration until it enters the sporangium, an organ that produces and contains the spores of *Pilobolus* spp. It gradually fills with water until it explodes, propelling the spores at a speed of 10.8 meters per second and a height of up to two meters. It has been reported that the spores and L3 of *Dictyocaulus* spp., housed in the sporangium, can be found at up to 2.5 meters from the dung; thus, facilitating the distribution of *Dictyocaulus* spp., in the pastures of the livestock production system. Studies have reported that *Pilobolus*-free plots reduce the elimination of *Dictyocaulus spp* L1 in feces up to 90% and decreases 85.02% of adult parasite burdens within the host (Biggane and Gormally, 1994). Therefore, the protection of dung beetles will enable the regulation of populations for both GIN and *D. viviparus* and, in addition, managing the germination of the fungus *Pilobolus* spp., favoring the epidemiological modulation of nematodes.

B. **Nematophagous nematodes:** *Butlerius* spp. is a nematode isolated from soils with decomposing organic matter. *In vitro* studies report reductions in the *H. contortus* population of 61.9% (Silva *et al*., 2017).

C. **Bacteria:** In recent years, one of the most evaluated bacteria for GIN control has been *Bacillus thuringenisis*, a gram-positive saprophytic spore-forming entomopathogenic bacteria used as a biocontrol agent. *Bacillus thuringenisis* sporulation process produces a "Crystal Protein," which has proven toxicity against various insects. The use of the toxin on *in vivo* trials (0.5 mg/kg BW IM) with small ruminants, has reported reductions in the populations of adult worms of *Haemonchus contortus* ranging from 50% to 70% (López *et al*., 2006). Other studies report doses of 20 mL PO/SD [10^{10} CFU/mL^{-1}] to reduce EPG up to 20% and to reduce L3 development in 95% (Nachtigall *et al*., 2022). It's important to note that the effectiveness of *Bacillus thuringiensis* against lungworms has not been well documented, and its use in this context may not be supported by scientific evidence.

D. **Nematophagous fungi:** *Duddingtonia flagrans*, a nematophagous fungus, has emerged as a promising biological control agent against GIN, with a dose-dependent effect in organic dairy goats (Maurer, 2021). *Duddingtonia flagrans* chlamydospores can survive the gastrointestinal passage of ruminants, germinate, and colonize fresh feces forming traps to capture GIN larvae (Torres-Acosta and Hoste, 2008). These traps adhere to the surface of the nematode, penetrate its cuticle, and grow an infectious bulb within the L3; from which,

trophic hyphae grow inside the body of the nematode and digest its content (GrØnvold *et al*., 1996). The use of nematophagous fungi has been proposed as an additive to the diet through nutritional blocks, pellets, and cookies. It has also been effective in reducing the GIN larvae population in calves under an organic milk production system (Mendoza-de Gives *et al*., 2018; Pérez, 2017), and EPG counts in cattle (Fernández, 2023, Jobim, 2008), goats (Vilela, 2020), and horses (Buzatti, 2015). Overall, reports suggest a dose of 1×10^6 chlamydospores (chs) kg/LW/PO 10 d, to obtain GIN L3 reductions of 57-84% in cattle, 84% horses, 86% goats (Mendoza de Gives *et al*., 2018; Ortíz-Perez *et al*., 2017; Assis *et al*., 2012; Dias *et al*., 2007). On the other hand, *in vitro* studies reported that a concentration of 600 chs/gh has the capacity to reduce *D. viviparus* larvae by 50.9% (Fernández *et al*., 1999). Most recent studies have reported its use in combination with *Caesalpinia coriaria* as an alternative to reduce EPG in sheep (Chavarría-Joya *et al*., 2022).

The use of biological control agents has shown promising results on *in vivo* trials. However, prior to its implementation, it must be ensured that the agent selected for biological control is harmless to the environment to avoid altering the natural balance of the ecosystem. Furthermore, biological control agents are edaphic fauna and can be used as a health indicator of the ecosystem, thus, by seeking the rational and conscious use of chemical products we can minimize their impact on the soil fauna and obtain additional benefits such as parasite control.

II. CONTROL OF INFRAPOPULATIONS. Refers to the control of the nematode stages found inside the host (XL3, young adults and adult parasites), which represents 20% of the parasite population within a Livestock Production System (LPS).

2.1. IMMUNONUTRITION. Immunonutrition refers to a nutritional support that uses special nutrients, like proteins, to modulate the immunological response to combat parasitic infection. Immunonutrition not only contributes to parasitic control but also holds promise in reducing reliance on anthelmintics and promoting sustainable livestock management. As the field of immunonutrition continues to evolve, understanding the intricate interplay between nutrition and immunity offers novel avenues for mitigating parasitic challenges in cattle production. One of the main tools that the host can use to counteract the negative effects of nematodes is immunity (Jackson and Waller, 2008); and it is necessary to remember that the immunological reaction against GIN also generates a nutritional cost associated with the following factors (Sykes, 2010):

a) Increase in the metabolic activity during the activation of immune cells, recruitment, and activation of leukocytes; and a double or triple increase in the consumption of oxygen, glucose and glutamine (Colditz, 2008).
b) Lower availability of nutrients due to anorexia and malabsorption syndrome caused by parasitic infections.
c) Modification in the physiological priorities for the use of nutrients, preventing their use by non immunological tissues.
d) Rapid production of immunity cells of protein origin.
e) Repair of the damage caused to the host's gastrointestinal tissue.

There are several factors playing a significant role in determining an animal's susceptibility or resistance to nematode infections, and nutrition is one of the factors leading the list. Proteins play a fundamental role in orchestrating the immune response, serving as key components of the immune system's structure, function, and regulation (Jackson, 2008). Houdijk *et al*., (2005, 2006) demonstrated that protein supplementation in ruminants influences the development of immunity against GIN. Amino acids, such as arginine and glutamine, are considered vital components of immunonutrition, influencing immune cell function and promoting an environment unfavorable to parasites. Studies by Gupta *et al*. (2019) and He *et al*. (2011) illustrate the immunomodulatory effects of specific amino acids, suggesting their potential as adjuncts in parasite control strategies. It has been reported, for calves offered with a higher percentage of metabolizable protein (MP) in their diet, to have lower rates of parasite establishment, less clinical signs, and less biochemical and hematological alterations (Gennari *et al*., 1995). Thus, protein supplementation in parasitized cattle allows them to improve their resistance and resilience and to help mitigate the impact of parasitic burdens (Houdijk, 2012; Jones *et al*., 2011; Hoste *et al*., 2008).

However, other elements like antioxidants, including vitamins C and E, also enhance the immune response and helps reducing oxidative stress associated with parasitic infections (Li *et al*., 2017; Pernthaner *et al*., 2005). Trace minerals, including selenium and zinc, play crucial roles in immune; research performed by Arthington *et al*. (2003) and Katsoulos *et al*. (2010) emphasize the importance of adequate mineral supplementation in promoting host resistance against parasites. Overall, authors recommend a balanced supplementation that provides both PM, EM and antioxidants (Li *et al*., 2017; Houdijk, 2012). Immunonutrition can be achieved either through the inclusion of commercial concentrates or the use of forage resources such as legumes, which have a high percentage of crude protein (20-26%), secondary plant metabolites like antioxidants, and optimal digestibility (70-80%).

2.2. MICROBIOME. The intricate relationship between the gut microbiome and nematode infestations in cattle has become a subject of growing interest due to its potential implications for sustainable parasite control strategies. Parasitic infections are known to induce dysbiosis and inflammation. Recent studies reported that susceptibility to parasitic infections by GIN in ruminants is partially mediated by the composition and/or functional alterations of the host's intestinal microflora (Cortés *et al*., 2020). The study of the ruminal microbiome (RMb) has been the focus of attention in recent years due to the constant search for alternatives to achieve food security and sustainability; and has been considered the key to success for productive performance, being associated with better development, feed conversion and increase in milk production (Raza *et al*., 2022). It has also been reported that RMb could regulate the immune response against GIN infections and respiratory diseases, and/or mitigate the negative impact (Colombo et al., 2021). Recent studies report that parasitic establishment generates an alteration in both the composition and functionality of the RMb within the host; it has been associated for some microbial communities to have an active role in the infection and establishment of GIN. While the post-ruminal microbiota (PRMb) has been directly associated with immune modulation in the intestinal mucosa (Cortés *et al*., 2020). Furthermore, it

has been reported that GIN infections generate an increase in *Proteobacteria* which affects carbohydrate transport and metabolism (Wang *et al*., 2022).

The use of prebiotics, non-digestible dietary components that selectively promote the growth and activity of beneficial gut microbiota, has emerged as a promising approach for GIN control in cattle. Prebiotics, including oligosaccharides and polysaccharides, selectively stimulate the growth and activity of beneficial microbiota (RMb and PRMb), such as *Lactobacilli* and *Bifidobacteria*. Enhanced populations of beneficial bacteria promote gut health and competition with GIN, leading to reduced parasite establishment and survival. Studies by Terrill *et al*. (2012) and Burke *et al*. (2010) have demonstrated the efficacy of prebiotics, such as fructooligosaccharides (FOS) and mannanoligosaccharides (MOS), in reducing fecal egg counts and GIN burdens in cattle. Furthermore, prebiotic supplementation has been shown to enhance immune responses, including increased production of mucosal immunoglobulins and cytokines, contributing to host resistance against GIN infections. Inulin and tannins have been proposed as prebiotics for livestock increasing resistance and/or resilience against nematode infections; due to the impact generated on the composition and functioning of the RMb and PRMb and to favor immune homeostasis at the intestinal mucosa level. Chicory (*Cichorium intybus*), a temperate climate legume rich in fiber and bioactive compounds, such as inulin, also promotes intestinal health, reducing populations of pro-inflammatory bacteria and increasing the proportion of populations considered beneficial (Valente *et al*., 2023; Roony *et al*., 2023). Although there are few studies with ruminants, the use of inulin in pig's diets reduced the number of adult parasites and fecundity of parasitic females of *Trichuris suis* and *Oesophagostomum dentatum*, respectively. It has been observed that the use of inulin can promote local Th2 polarization and the integrity of the colonic mucosa. Likewise, inulin increases beneficial microbiota (*Lactobacillus* and *Bifidobacterium*) and reduces the pro-inflammatory microbiota (*Proteobacteria* and *Firmicutes*) (Roony *et al*., 2023). Another promising alternative is the use of tannins; for several decades the use of condensed tannins as nutraceuticals has been reported due to their capacity to form tannin–protein complexes that render the latter unavailable to ruminal bacteria, thus, promoting their absorption as a bypass protein, and thereby, increasing amino acids and peptides absorption in the small intestine (Hoste *et al*., 2008). This in turn improves mucosal immunity against parasitic infections. It has been shown that condensed tannins can modulate MbR in small ruminants, favoring the growth of beneficial populations such as *Lactobacillus* and *Bifidobacterium*; and to increase total ruminal SCFAs (acetate, butyrate, valerate and isovalerate) (Roony *et al*., 2023).The most recent studies conclude that increased levels of fermentable non-starch polysaccharides in refined diets compromise immunity to helminth infection; while the manipulation of diets integrating the use of prebiotics (tannins, polyphenols, and inulin) generates significant changes in RMb and PRMb that benefit the host. Therefore, the use of prebiotics offers a sustainable approach for GIN control and could be considered a new strategy to promote resistance and/or resilience to infection by enteric parasites (Valente *et al*., 2023; Roony *et al*., 2023).

2.3. VACCINES. According to the World Health Organization (WHO), a vaccine is "*any preparation intended to generate immunity against a disease by stimulating the*

production of antibodies." For a vaccine to be considered effective, it is necessary that the number of parasites within the host is reduced and/or that their establishment is decreased, which can be achieved either by inhibiting the establishment of infective L3 larvae, or by increasing the mortality of already established adult parasites (Ketzis *et al*., 2006). During the last 20 years, various vaccines focused on GIN control have been developed. However, to date, the development of a viable vaccine against GIN has not been achieved. On the contrary, vaccines designed to control dictyocaulosis have shown the ability to reduce both the number of adult parasites by 93.3% and L3 in feces by 93.5%. Animals exposed to *D. viviparus* can mount a good immune response between 8- and 11-days post-infection (dpi) and the patent period. However, immunity decreases in the post-patent period and it's lost after 6-12 months post infection (pi), animals being left fully susceptible to parasitic establishment, while maintaining immunity against the development of adult parasites. The vaccine has been designed to strengthen the defenses against larval establishment in the host and is exposure dependent; it consists of live-attenuated infective larvae (L3va) 1000-2000 L3va/PO 0-4 weeks, which produces an infection that will not reach patency, and so, the immune response generated is very similar to the immune response obtained from a low natural infection. However, it is worth mentioning that the vaccine has been removed from some international markets since immunity is not maintained, it has high costs, a short shelf life, and requires a cold chain.

2.4. USE OF BIOACTIVE PLANTS. Research in ethnoveterinary medicine focuses on the scientific validation for the safety of use and effectiveness of traditional medicine to treat various diseases in animals (Barrau *et al*., 2005). The hypothesis of the possible anthelmintic effect of plant secondary metabolites (PSM) initially arose after observing that small ruminants grazing in grasslands with temperate legumes had a lower EPG´s (Hoskins *et al*., 2000; Niezen *et al*., 1995). In the first instance, it was proposed that the anthelmintic effect was indirect through immunonutrition (See section 2.1) (Coop and Kyriazakis, 1999). However, multiple *in vitro* studies have reported that some PSM bind to structural proteins of nematodes and thereby affect their biology and/or reproduction (Hoste *et al*., 2006). Various studies carried out in the last decade have shown that there is a broad group of bioactive PSM that affect nematodes and whose mechanism of action is not limited to binding structural proteins. Currently, the mechanisms of action of PSM reported include: binding to structural proteins of adult parasites (the cuticle, oral cavity, esophagus, cloaca and vulva, which are proline-rich structures, an amino acid with affinity to form stable complexes with tannins) (Hoste *et al*., 2006), effects on cuticle (degradation of structural collagens and collagen-like proteins), larval and adult parasite motility (affectation of sarcomeres), larval exsheathment (structural and ultrastructural alterations in the sheath-cuticle complex), egg hatching and embryonic development, as well as in the reduction of EPG (von Son de Fernex *et al*., 2023, 2019, 2016, 2015; Brunet *et al*., 2011; Alonso Diaz *et al*., 2008; Brunet *et al*., 2008; Heckendorn *et al*., 2007; Hoste *et al*., 2006; Rojas *et al*., 2006; Paolini *et al*., 2003; Athanasiadou *et al*., 2001). Plant secondary metabolites are allelochemical compounds which are not directly related to their growth, development, or reproduction; but are part of their chemical defense for environmental interaction. Many of these bioactive compounds are responsible for the coloring, flavor, or fragrance of plants (Adak and Kumar, 2022; Sepúlveda-Jiménez *et al*., 2003). Plant secondary metabolites are synthesized and/or

accumulate in the plant in the presence of damage or injuries caused by: i) biological agents (bacteria, viruses, parasites or fungi), ii) chemical agents (bactericides, fungicides, insecticides, nematocides), iii) situations of climatic stress (solar radiation and/or herbivory, arthropods and vertebrates), iv) environmental adaptation (attraction of pollinators and dispersers) and, v) cellular and tissue repair. There are over 20,000 structures of PSM, which have been widely used in the cosmetic, food, and pharmaceutical industries (Ávalos-García and Pérez-Urria, 2009). Both *in vitro* and *in vivo* studies assessing the AH potential of plants have facilitated the identification and isolation of various PSM with AH
like activity against various genera of GIN and against *D. viviparus*. Within these, some of the most reported PSM groups with anthelmintic activity are:

A. **Terpenes:** Terpenes are derived from the fusion of five carbons, a functional unit known as isoprene, and are classified according to the number of isoprene units, as: monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, quinones, etc., there are over 40,000 molecules reported (Ávalos-García and Pérez-Urria, 2009). Within the wide range of biological activity, the anthelmintic effect of some phytochemicals belonging to this group like pyrethrins, monoterpene, cucubitacin, triterpen-4-ol, borneol, β-elemene, sesquiterpene lactones; have been reported to affect the nervous system of insects (Adak and Kumar, 2022; Peña-Espinoza *et al*., 2016; Sepúlveda-Jiménez *et al*., 2003).

B. **Phenolic compounds.** Phenylpropanoids are molecules that have an aromatic ring with one or more hydroxyl groups in their structure. They are classified according to the number of rings, hydroxyl groups and the position of the phenolic rings they have. Within this group, some of the reported bioactive compounds with AH-like activity are: coumarins, caffeic acid, quercetin, rutin and various flavonoids, monophenols (cresol, thymol, carvacrol), condensed tannins (epigallocatechins, catechin), derivatives of cinnamic acid (p -coumaric, caffeic acid, ferulic acid) (von Son-de Fernex *et al*., 2023; Adak and Kumar, 2022; Peña-Espinoza *et al*., 2016; Hoste *et al*., 2012; von Son-de Fernex *et al*., 2012; Alonso-Diaz *et al*., 2008; Barrau *et al*., 2005; Sepúlveda-Jiménez *et al*., 2004).

C. **Glycosides**. Metabolites made from a sugar molecule condensed with another molecule that contains a hydroxyl group. Within this classification we can find saponins (inhibit acetylcholinesterase causing paralysis of parasites), cardiac glycosides, cyanogenic glycosides and glucosinolates. This group is characterized by possessing detergent properties and having a powerful AH-like activity, like the cardenolide, which affects the transport of sodium-potassium ions in helminths (Adak and Kummar, 2022).

D. **Alkaloids.** Heterocyclic compounds synthesized from amino acids whose toxicity has been directly associated with their ability to block neuroreceptors due to the similarity they have with several neurotransmitters. There are more than 15,000 molecules reported with biological activity, within which, some have AH-like activity: i) matrine and sparteine, which have the capacity to affect the motility of helminths, ii) mimosine, dicethrin and sanguirenin, affect

acetylcholine receptors by inhibiting glucose uptake (Adak and Kummar, 2022; Sepúlveda-Jiménez *et al*., 2003).

Likewise, although scarce, some *in vivo* studies have validated the use of plants such as *Gliricidia sepium*, Lucerne (*Medicago sativa*), and Sulla (*Hedysarum coronarium*) as sustainable alternatives for the prevention and control of nematodes in cattle (von Son -de Fernex *et al*., 2018; González-Torralba, 2017; Peña-Espinoza *et al*., 2016; Molan *et al*., 2003; Hoskins *et al*., 2000). The use of plants as a nutritional supplement has been proposed as a strategy that allows both, to mitigate the effect of forage seasonality and to add nutraceutical value. A nutraceutical is considered a food or a part of it, which in addition to nourishing the animal, has medicinal properties or health benefits such as the prevention and/or treatment of diseases. However, one of the main limitations of nutraceutical with AH-like activity inclusion in parasite control schemes is that the effect will fully depend on the nature and concentration of PSM´s, as well as whether voluntary intake is for a prolonged period (Rmdani *et al*., 2023; Hoste *et al*., 2015).

Finally, among all the benefits previously described the inclusion of bioactive plants in a LPS will also allow Zoopharmacognosy to occur naturally. Zoopharmacognosy is the term used to describe animal's ability to self-medicate through the voluntary intake of specific plants or substances that provide a beneficial effect on health (Villalba and Landau, 2012; Villalba *et al*., 2010). Even though there is scarce information in the context of nematode infections in cattle, self-medication behaviors have been observed, suggesting an innate ability of animals to manage parasitic challenges through different mechanisms like chemoreception, where animals may use olfactory and gustatory cues to identify plants containing bioactive compounds with anthelmintic properties. Some studies have reported selective grazing of cattle on specific plants, such as tannin-rich forages or browse species, coinciding with periods of high GIN burden (Hoste *et al*., 2016; Waghorn *et al*., 2003; Athanasiadou *et al*., 2001); which is consistent with several cafeteria trials reporting higher replacement rates and preferences of parasitized cattle for the intake of certain tropical plants with bioactive properties (Espinosa-Garrido *et al*., unpublished data; González-Arcia *et al*., 2014). However,
further research is needed to expand knowledge on regards of both bioactive plant species and SPM with AH-like activity, as well as its efficacy and safety, to promote their implementation within LPS for cattle self medication. Livestock self-medication against nematode infections highlights the innate capacity of animals to manage parasitic challenges; understanding and leveraging these behaviors, holistic and sustainable approaches to parasite control in cattle populations could be developed and/or improved.

CONCLUSION.

Gastrointestinal nematodes (GIN) and *Dictyocaulus viviparus*, commonly known as lungworm, pose significant challenges to animal health and performance in livestock, affecting both national and farm economy. Basic knowledge of the biology and ecology of parasitic diseases within a Livestock Production System can determine the success or failure of a parasite control scheme. Among the various challenges facing the control of parasitic diseases in cattle, we can find pharmacological resistance,

sustainability, the increase of market demand for products and by-products of animal origin raised under ethical standards, animal health and welfare, as well as free of chemical residues. The strict rules of organic farming, including limited use of medical drugs, can pose challenges for animal health; however, organic livestock production has been found to meet the demands of consumers who are critical of conventional methods, and in some regions are closely aligned with traditional practices. Despite these challenges, organic livestock farming has been found to have the potential to meet high standards of animal health and welfare. Understanding of consumer expectations and a commitment to sustainability and animal welfare are essential for fostering resilience and long-term success in the livestock industry. Therefore, it is imperative to know the different parasite control alternatives available today to promote healthy ecosystems and understanding that consumer preferences are crucial for fostering sustainable and ethical practices that align with changing market demands. Even though the most appropriate method or parasite control scheme may vary among farms and regions, the studies presented in this summary collectively underscore the importance of a multifaceted and sustainable approach to helminth control in ruminants.

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Dietary improvement of fertility in dairy cows

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In sustainable farming, dairy cattle need to produce calves at regular intervals. One major obstacle to the sustainability of dairy farms is the high culling rate due to infertility/subfertility caused by or as a consequence of negative energy balance during the post-partum period. I will present studies that have tested the impact of nutritional management of dairy cows focusing on overcoming the energy deficit and how it affects the metabolic hormones that regulate ovarian activity and folliculogenesis, and the microenvironment of ovarian follicles, oocyte quality and developmental competence to form an embryo, uterine function and pregnancy rate. These include in vivo studies which analyzed the impact of energy level on insulin, IGF-1 and changes in IGFBPs on follicular dynamics, resumption of ovarian activity during post-partum period and pregnancy and live birth. In parallel, we assessed the developmental competence of the oocytes from cows that received different energy sources before ovum pickup and analyzed oocytes grade and development after in vitro maturation and fertilization. We determined that diets containing dietary fats improved oocyte quality and development. However, glucogenic diets which increase insulin and IGF-1 and accelerate ovarian activity folliculogenesis and ovulation, may not be necessarily good for oocyte quality and development. I will also present the results of complementary in vitro studies which analyzed the impact of dietary sources of polyunsaturated fatty acids on oocyte maturation and development to blastocyst stage after in vitro fertililization. Following this, I will present results of the experiments which we analysed the impact of polyunsaturated fatty acids (PUFAs) on health and the developmental competence of the oocytes and uterine function. We show the differential effects of Omega 3 fatty acids as compared to Omega 6 PUFA on cytoplasmic regulators of oocyte maturation including MAP kinases and cAMP and mitochondria distribution and function within the oocyte. The impact of PUFAs on offspring gender, uterine immunity and receptivity and prostaglandins will also be discussed. Results from these studies have provided new insight into nutritional management of fertility in dairy cows and clarified the impact of dietary energy sources particularly fat composition of diet in these processes.

Smart nutrition: improving the quality of ruminant milk fat

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Over the past 20 years, consumers have increased their knowledge about the nutrients they get from food. This has been reflected in a greater demand for high-quality foods. In the specific case of dairy products, one of the components that has had the most criticism and, above all, the one that has been the most in the debate, is milk fat. This is due to its high content of saturated fatty acids, which in some studies has been linked to human health problems.

Researchers have taken the task of reducing the concentration of saturated fatty acids in dairy products by modulating the diet of ruminants. In this sense, in this presentation, some studies related to the modulation of diets of goats, sheep and dairy cows will be described to increase the number of fatty acids with a positive effect on human health. These studies have been carried out to analyze the effect of including different feed inputs to improve lipid quality of milk, cheese, and ice cream.

Finally, in addition to improving the dairy fatty acid profile, it has also been shown that enteric methane production is reduced in some cases. Therefore, today, more consistently, not only the improvement of lipid quality is sought but also the effects of diet on the environment.

Education in ruminant veterinary medicine and production

Challenges and Opportunities in Veterinary Medical Education to meet the needs of Food Animal Producers

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Veterinarians are critical to the success of rural communities and economies around the globe. Veterinarians support food and fiber animal industries, maintain the health of companion animals, identify zoonotic diseases, and play a critical role in disease surveillance. Food supply veterinarians play essential roles in animal health and welfare, as well as food safety and security across the animal agriculture supply chain. The demand for veterinarians and other veterinary team members is notable in nearly all areas of veterinary medicine from private practice to academia to government service. Of particular concern is the need for food supply veterinarians.

The complicated challenges surrounding the shortage of food supply veterinarians are noted as are the unique challenges of delivering a veterinary medical education. Strategies and innovative programs are needed to support and prepare veterinary students, recent graduates, and employers. Educational approaches to expand veterinary instruction beyond individual diagnosis and treatment are warranted. Focusing on areas such as production medicine, integrated services, practice management, leadership, professional support, and personal health for veterinarians has the potential to recruit and retain more graduates in food supply medicine thereby supporting animal agriculture and its producers.

The presentation will address 1) *Education of students beyond traditional curricular offerings* 2) *Instructional strategies to mitigate food-supply veterinary shortages* 3) *Development of continuing education programs for graduate veterinarians learning in tandem with their future associates.*

The goal of the presentation is to consider options for innovative educational approaches to alleviate food supply veterinary shortage. Strategies, approaches, and templates will be shared focused on programs intended to address food supply veterinary shortage and create improved sustainability for animal agriculture.

ORAL PRESENTATIONS

Infectious diseases

1055 - Bovine brucellosis: Advantages of using vaccine Brucella abortus RB51

Author: Luis Samartino, Pablo Martino, Josué Lorenzatti, Geert Vertenten

Objectives

Bovine brucellosis is a worldwide disease and still causes severe problems. In cattle, two vaccines are available: Strain (S)19 and strain RB51. Both induce similar protection; however, RB51 does not induce antibodies which interfere with diagnostic results and can be applied more than once without causing inconvenience.

We show here some examples of the contribution of the strain RB51 for controlling brucellosis in infected dairy farms.

Material and methods

Two dairy farms were selected for this study:

Farm A of 2224 animals with an initial brucellosis prevalence of 1.75%, and farm B of 510 animals with an initial brucellosis prevalence of 3.1%.

Strain 19 was administered to all female calves between 3 and 8 months of age in both farms.

Both herds had abortions and positive animals by serology. Therefore, a testing and culling program was performed. In both cases, positive animals were removed from the herd as soon as they were detected.

Brucella abortus *Strain [Bovilis RB51, MSD Animal Health] Vaccine* was used as adult vaccination.

For serology, three diagnostic tests were applied: Buffer Plate Antigen Test, Complement Fixation and Fluorescent Polarization.

Results

In this study, two examples from infected dairy herds are shown, where female animals were S19 vaccinated as calves. After 6 months of completing the test and slaughter program, results were very poor. Positive animals were found in both herds. Besides, abortions were keeping the infection active. *B. abortus* biovar 1 was isolated from placental tissues and milk in both farms. For this reason, we decided to apply Bovilis RB51 to the whole herd. No abortions were seen after application to pregnant animals. In earlier studies, we demonstrated that Bovilis RB51 applied to animals previously vaccinated as a calf with either S19 and/or Bovilis RB51 rarely abort after being vaccinated as adults. Management of both herds did not change after Bovilis RB51 application and no other tool was applied. In one of the farms, due to the success of the Bovilis RB51 vaccination, we decided to apply a second Bovilis RB51 dose after a year to the whole herd to prevent future complications with the disease. Serology continued to be negative in both farms after the second Bovilis RB51 administration.

After six months using Bovilis RB51, both herds were considered brucellosis free.

Conclusions

Strain 19 and RB51 are both good vaccines; the advantage of Bovilis RB51 is that this vaccine can be used anytime without interfering with diagnostic results, it doesn't induce abortion in pregnant animals previously vaccinated as a calf, and, moreover, it is an excellent tool to control bovine brucellosis.

1127 - Effectiveness and safety of brucellosis control using the RB51 vaccine in adult beef cows

Author: João Drumond, Carlos Fernandes, João Viana, Ana Cristina Figueiredo, Gustave Pereira, Henderson Ayres, Lais Vieira, Denis Antônio, Geert Vertenten, Luis Ernesto Samartino

Objectives

Brucellosis is a disease of global importance since few countries have managed to eradicate it. In cattle, brucellosis causes significant losses, mainly by an increase in abortions and thus by reducing herds reproductive performance. The RB51 vaccine has been successfully used in programs to control brucellosis outbreaks by mass vaccination of herds in countries such as Brazil. Vaccination of adult cows with the RB51 strain is an alternative to reduce the spread of the pathogen in commercial herds. However, vaccination strategies still need to be evaluated. Thus, the aim of this study was to compare the effectiveness brucellosis control and reproductive performance using one or two doses of RB51 (Bovilis RB51, MSD Animal Health) (given at 6-months interval) in commercial beef farms with adult females previously vaccinated with S19 at 3-8 months of age.

Material and methods

This experiment was conducted at two commercial beef farms (1 and 2) in Minas Gerais State, Brazil, from September 2020 to June 2022. These farms were selected because of records of endemic brucellosis problem and a prevalence of 6 to 8% of positive cows. Their regular vaccination protocol with the S19 vaccine included only female calves between 3 to 8 months of age. Farm 1 (N=477) used fixed-time artificial insemination (FTAI), with inseminations and births occurring throughout the year. Farm 2 (N=673) adopted a breeding season from December to March, with one FTAI followed by natural mating with bulls. An initial serology screening (Day 0) was carried out in all adult females using Rose Bengal test (RB). Cows with positive results were re-evaluated using a Slow agglutination test (2-ME, 2-mercaptoethanol), and those positive in this test were discarded. If the result of the 2-ME was negative, they were re-incorporated into the herd. Non-reactive cows, in each farm, were then randomly assigned into three experimental groups, balanced for parity, days of pregnancy and BCS: G1 (N=277), a single vaccination with RB51; G2 (N=283), two shots of RB51 given 6 months apart; and G3 (N=279, control group), no treatment. Within each herd, all cows were kept together and under the same management. Serological (RB) and 2ME tests were carried out on Days 90, 180, 270 and 360. In each evaluation the positive cows (as confirmed with 2ME) were discarded. The variables were analyzed using the SAS software (SAS Institute Inc., Cary, NC), at 5% of probability.

Results

Seropositive cows were still detected in both vaccinated and control groups at 90 days (1/513 and 1/312 for G1 and G3, respectively). However, no new infections, as indicated by negative seroconversion in the 2-ME tests, were detected in G1 at 180 days or in G1 and G2 at 270 and 360 days, whereas new seropositive cows were diagnosed in all exams in G3 (control). Therefore, the cumulative number of new infections was lower in vaccinated than in control cows [0.36^{b} (1/274), 0.00^{b} (0/279) and 3.65^{a}% (10/274) for groups G1, G2 and G3 respectively; $P<0.05$]. The percentage of seropositive cows at the RB test was similar ($P=0.154$) among groups throughout the study. These results suggest that vaccination with either one or two doses of RB51 did not lead to positive reactions by the RB or 2-ME tests. There was no difference ($P=0.233$) among groups in the partum to first AI interval. However, in farm 1 the number of days open was greater in the control than in vaccinated groups (79.7 ± 14.2^{a} and 77.2 ± 13.7^{a} vs 85.5 ± 15.9^{b} days for groups G1, G2 and G3, respectively; $P=0.043$), and in farm 2 the pregnancy rate was lower in the control than in the group vaccinated once (89.1%a, 85.0%ab and 80.1%b for groups G1, G2 and G3, respectively; P=0,041).

Conclusions

Vaccination with RB51 (Bovilis RB51, MSD Animal Health) in adult beef cows, previously vaccinated with S19 at 3-8 months of age, managed in an extensive regime, reduces the occurrence of new cases of brucellosis, that is, the spread of the pathogen in herds. Additionally, vaccination improves the reproductive performance of immunized cows. There are no indications that a second immunization, six months after the first, brings additional benefits in terms of protection or reproductive efficiency.

1200 - Prevention of digital dermatitis using bacterial complex-based solution

Author: RAPHAEL GUATTEO , Réhane Chinon-Goron, Gino Scimia, Alexandre Brame

Objectives

Lameness is a major threat to the welfare of cattle, farmers and the profitability of cattle farms. Among the causes of lameness, digital dermatitis (DD) is one of the most frequent. In addition, once it has been introduced into a farm, its eradication is illusory. Among the control strategies employed, collective treatment solutions, via foot baths or directly by spray on the hooves, is common practice. However, several of the products used (such as formaldehyde or copper sulphate) are toxic to humans or the environment, and there is little evidence that the other products are effective. Finally, today's society expects a reduction in the use of chemical agents in animal husbandry. Since digital dermatitis results from colonization of the foot by various bacterial complexes (including treponemes), it is reasonable to think that applying a barrier flora could help to control digital dermatitis. Therefore, this study aimed to assess the effect of a bacterial complex-based solution applied directly to the feet of cattle, compared with no treatment, in preventing the occurrence or reducing the persistence of digital dermatitis (DD) lesions.

Material and methods

This study has received the favourable opinion of an ethics committee (CERVO 2023-9-V). This study was conducted in 5 dairy herds (mainly Prim'holstein) and 640 dairy cows (434 multiparous and 206 primiparous cows). The mean initial prevalence of DD lesions (M1, M2, M3, M4, M4.1) was 32,6% and 32% respectively for the right and left feet. The statistical unit of interest was the foot. To reduce the sample size required and to avoid a too-large herd effect, it was decided to consider the cow as its control. Thus, in each farm, the left foot of the animals received an application of the bacterial complex once a week (without pre-washing the skin foot) for 6 months, while the right foot was left untreated and served as a control. At each monthly visit, the presence of any DD lesions (based on the M5 scoring system) was checked in the milking parlour using a swivelling mirror and any risk factors for the occurrence or persistence of DD were noted at every visit. The effect on the occurrence or persistence of DD lesions was assessed using survival analysis methods and the Cox model.

Results

Finally, our findings demonstrated that feet treated with bacterial complex had a significantly lower incidence (HR=0.77 [0.62-0.96]) than untreated feet (ref). Besides the application of the bacterial complex, the presence of a contralateral lesion (HR=2.26 [1.80-2.86]) and the stage of lactation (>150 DIM HR=2.60 [2.00-3.37]) also significantly increased the risk of a new DD lesion. The study showed that 50% of healthy feet at the start of the study developed a DD lesion after 90 days for untreated feet and 150 days for feet treated with the bacterial complex. Application of the bacterial complex significantly delayed the occurrence of DD lesions by a mean of 29 days ($p<0.05$). There was therefore no effect on the persistence of lesions (prevention, not cure).

Conclusions

To our knowledge, this is the **first study investigating and reporting a significant preventive effect on the occurrence of digital dermatitis lesions in dairy cows based on using a complex of bacteria** directly applied to the feet. This type of biocontrol product offers an alternative to conventional crop disinfection products and means that the use of chemical inputs can be reduced.

1203 - Rapid and efficient BVDV control in a large industrial dairy farm using various diagnostic toolkits in Hungary, Central Europe

Author: Attila Dobos, Vilmos Dobos, Istvan Kiss

Objectives

Bovine viral diarrhea virus (BVDV) is endemic in several countries and can have a major impact on cattle health. While EU countries have introduced either voluntary or mandatory BVD eradication programmes throughout the last decades, there is no mandatory national BVD eradication programme in Hungary. There are several industrial dairy herds in Hungary having more than 1000 dairy cows, and the concentration of the Hungarian dairy population is one of the highest one all over Europe. Since the cornerstone of the epidemiology of BVD is the presence of persistently infected (PI) immunotolerant calves, born because of the infection of susceptible dams during the first trimester of gestation, these concentrated animal populations, with many cows being in the susceptible gestation period, are at a reasonably high risk for BVDV infection. Such scenario calls for the efficient identification and quick removal of the detected PI animals in herds embarking on BVD control, as the presented study demonstrates. The proper immune status of the herd, and the pregnant cows in particular, was ensured by vaccination with a modified live vaccine of proven cross-subtype efficacy.

Material and methods

The large industrial dairy farm embarked on the control of BVDV starting in January 2023. The total herd size was 1571 Holstein Friesian cattle (850 milking cows and 721 heifers). The herd never vaccinated before against BVD and no animals entered the farms throughout the investigation period. Serum samples were screened by a commercial qPCR, and the subtyping of the detected BVDVs was performed by the partial nucleotide sequences of the Npro coding genomic region. Follow up monitoring investigations of the newborn calves were performed by serology (commercial ELISA) and qPCR between January - November 2023.

Results

Following the foundational steps of eradication, we first assessed the BVDV status of the entire herd (n=1571), resulting in the identification of 21 PI animals. These were promptly removed to eliminate the direct sources of viral infection and preventing further spread. Simultaneously, the farm began using a live, attenuated BVDV vaccine (Mucosiffa, Ceva-Sante Animale, France), applied concurrently to the entire herd, with the aim to provide efficient fetal protection for the pregnant animals. Throughout the investigation period 479 newborn calves were investigated by qPCR and ELISA, and 28 (5.84%) PI animals were found and removed. Pestvirius A subtype 1b was detected in the farm, and selected viruses were submitted to virus isolation, which revealed the presence of both biotypes of the virus, indicating pre-existing mucosal disease conditions on the farm. Sera originating from naïve cows from a BVDV-free herd, pre-immnunized by the Mucosiffa vaccine were used in cross-virus-neutralization test demonstrated an average titer of 1:141 and 1:430 against a ncp and a cp isolate, respectively, while it was 1:2110 against the homologous Oregon C24V subtype 1a strain. It is of note that antibody titres ≥1/20 were reported as cross-protective.

Conclusions

Our study demonstrated the utility of the whole herd screening and tight follow-up monitoring for fast and efficient identification and elimination of PI animals, which, supplemented by proper vaccination, led to the timely control of BVD in a large dairy farm, infected by a heterologous subtype of BVDV. However, it must be emphasized that all these measures work if stringent biosecurity measures are in place.

1423 - Prevalence and spatial distribution of infectious diseases of dairy cattle in Ontario, Canada

Author: Diego Nobrega, David Kelton

Objectives
Here we investigated the prevalence and spatial distribution of selected pathogens associated with infectious diseases of dairy cattle in Ontario, Canada. The cross-sectional study surveyed bulk tank milk for antibodies against bovine leukemia virus (BLV), *Mycobacterium avium* ssp. *paratuberculosis* (MAP), and *Salmonella* Dublin, and for the presence of mastitis pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*). Our results serve as a foundation for future work on disease trends and will inform the development of appropriate disease control strategies at the province level.
Material and methods
The Agriculture & Food Laboratory (AFL; Guelph, ON) tests BTM samples from all commercial dairy farms in Ontario for regulatory and payment purposes as part of the provincial Dairy Food Safety Program under the Ontario Milk Act. With permission, we obtained bulk tank milk (BTM) samples from every commercial dairy farm in Ontario (n = 3,286) between October 2021 and June 2022. Samples underwent ELISA testing for presence of BLV, MAP and *S*. Dublin antibodies, and quantitative PCR testing for the detection of specific antigens of pathogens associated with mastitis. Bayesian models were used to estimate prevalence considering imperfect test characteristic and previous results. We also carried out spatial analysis out to identify regional clusters of high pathogen prevalence.
Results
Prevalence varied for different pathogens. BLV was widespread across dairy farms in Ontario, with an estimated prevalence of 88.3%. Prevalence of MAP, *Staph. aureus* and *S*. Dublin in Ontario dairy herds were 39.8%, 31.5% and 5.1%, respectively. The vast majority of dairy herds in Ontario were free of intramammary infections caused by *Strep. agalactiae* and *M. bovis*. Clusters of increased test positivity rates were detected for *S*. Dublin, MAP, and *Staph. aureus*, indicating potential geographic risk factors for pathogen transmission. For *S*. Dublin, an area of increased test positivity rates was detected in southwestern Ontario, close to the Canada-US border where most of the dairy herds in Ontario are located. Conversely, a localized cluster of positive test outcomes involving 14 subdivisions located in the southeastern region of Ontario was detected for *Staph. aureus*. For MAP, we first identified an area in western Ontario with elevated test positivity rates, where the risk of a positive test result is 1.3 times that of the remaining study areas. Given that the western region of Ontario is responsible for almost 50% of all cattle movements within the province, local cattle purchases are common among farmers, and introducing animals from external sources predicts MAP herd positivity.
Conclusions
We described the prevalence and spatial distribution of BLV, MAP, *S*. Dublin, and pathogens causing contagious mastitis in Ontario. These results provide a foundation for future research and the development of disease control strategies at the provincial level. Herds positive for *S*. Dublin tended to be located in southwestern Ontario, and we hypothesize that cattle movement will be a risk factor for *S*. Dublin and MAP ELISA positivity. Our findings support that the prevalence of MAP increased in Ontario in recent years, with nearly half of the provincial herds considered infected. Likewise, our investigation demonstrates that BLV is widespread in Ontario, consistent with findings from other Canadian provinces. Conversely, the vast majority of dairy herds in Ontario is likely free of mastitis caused by *Strep agalactiae* and *M. bovis*. Finally, nearly a third of dairy farms will contain cows infected by *Staph. aureus*. Findings from our survey highlight the importance of the testing of aggregated samples and spatial analysis as part of disease surveillance programs and for implementing risk-based trading approaches among dairy producers.

1132 - Bluetongue serotype 3 outbreak in cattle in the Netherlands in 2023: Clinical signs, pathological findings, transmission and implications

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Objectives

Bluetongue (BT) is an infectious, non-contagious vector borne disease that is caused by bluetongue virus (BTV) and is transmitted by midges of the genus *Culicoides* spp. BT causes clinical disease and mortality particularly in sheep, although other ruminants are susceptible. Clinical signs are the result of endothelial damage by BTV and as a consequence oedema, haemorrhage, muscular degeneration and necrosis occur. The objective of this study was to provide an overview of the BTV serotype 3 outbreak in the Netherlands in 2023 by reporting the clinical manifestations, pathological findings, transmission and potential implications for cattle.

Material and methods

Clinical signs indicating BT were first reported on 3 September 2023. Two veterinary practices located in the middle of the Netherlands notified on five farms sheep with clinical signs indicative for BTV to the Dutch Food and Consumer Safety Authority (NVWA). A farm visit with a team of veterinary experts (Royal GD, NVWA and practitioners) was performed on 4 September and blood samples submitted for BTV testing to the reference laboratory Wageningen Bioveterinary Research (WBVR) that confirmed BT on 5 September on four of the suspected farms. On 8 September WBVR determined, through whole genome sequencing, BTV serotype 3 as most likely serotype which was later confirmed by the European Union Reference Laboratory (EURL) in Madrid.

To investigate whether the initial outbreak started in the middle of the Netherlands or whether BTV was already widespread present before the index cases were notified, thousand bulk milk samples that were submitted four routine investigation to Royal GD in August 2023 were analysed. These samples were randomly selected from twenty different geographical locations in the Netherlands and screened for the presence of BTV-antibodies. Data on confirmed and notified cases from the NVWA, mortality from the identification and registration data and milk production data were analysed to obtain a first impression of the impact of BTV-3.

Results

NVWA data up to 5 December 2023 showed that infections with BTV-3 were confirmed on 1,885 sheep farms and 1,919 cattle farms. In addition, symptoms consistent with BT have been reported at additional 1,463 cattle or sheep farms, in which BTV-3 has not (yet) been confirmed with PCR. A retrospective study using bulk milk indicated no signs of massive spread of BTV before early September.

Clinical signs in cattle included fever, lesions on the nose and in the oral cavity, nasal discharge, conjunctivitis, salivation, reduction of milk yield and lameness with swelling of the coronary band. Histopathological examination of affected mucous membranes revealed ulceration, subepithelial microbleeding, oedema, vasculitis, microangiopathy, and microvascular thrombosis. Additionally, a clear and significant drop in milk production was observed, which was most prominent in areas that were infected in September 2023. Data-analysis on I&R-data revealed an increased mortality in cattle. In three aborted calves, BTV-3 was detected in the spleen, with no congenital malformations. The course, duration of the disease, the number of sick cattle per farm, and the severity of symptoms in cattle varied widely.

Conclusions

In 2023, BTV-3 was detected in an early phase by the Dutch monitoring and surveillance system, and has spread rapidly over all twelve provinces in the Netherlands. To date, BTV-3 has also been confirmed in Belgium, Germany and the UK.

Although sheep exhibit the most severe clinical signs and highest mortality rates, also a large impact in cattle was observed. Both the clinical manifestation of BTV-3 and the severity varied per cow and per farm. Further investigations are being conducted to explain these differences and to prepare for the upcoming vector season in 2024.

1294 - Pain relief therapy for managing viral vesicular diseases and their consequences

Author: Peter Windsor Andrew, Delia Lacasta

Objectives

Review the emerging use of a novel topical pain-relief therapeutic approach to improve livestock welfare in outbreaks of viral diseases of livestock characterised by oral vesicular lesions. This provides a more appropriate alternative to use of antibiotic therapies, reducing antimicrobial use (AMU) concerns and resistance risk (AMR).

Material and methods

Clinical field trial evaluation of the application to oral viral vesicular lesions, of a spray-on therapeutic formulation that was developed for wound management during aversive animal husbandry procedures, containing two topical anaesthetics (lignocaine, bupivacaine), adrenalin and cetramide in a gel matrix (Tri-Solfen and Multi-Solfen, Medical Ethics, Australia; TS). Whilst the concept was conceived in 2018, first applications of the product at doses of between 0.5 to 10mls applied by spraying directly on lesions, occurred ininitially in outbreaks of: Foot-and-Mouth Disease (FMD) in cattle and buffalo in Laos and Cameroon in 2019; then Contagious Ecthyma (orf infections) in sheep in Spain in 2021; followed by Epizootic Haemorrhagic Disease in cattle in Spain in 2023.

Results

The first trial occurred in April 2019 when the peoduct was applied to oral and feet lesions in an outbreak involving FMD-affected cattle and buffalo (n = 136) in Laos. The immediate positive clinical impacts of improved demeanour and retrun of appetite and locomotion, impressed the farmers, vets and the animal health authorities, with registration of the product for FMD therapy soon occurring in Laos. Then, in November 2019, clinical responses and recoveries of FMD-affected cattle was conducted during an outbreak in Cameroon, comparing responses to treatments applied to each of three equal cohorts (n = 12), including: (i) the application of the TS to oral and foot lesions; (ii) the administration of parenteral oxytetraycline commonly used for FMD in Cameroon and other countries; and (iii) an untreated control group. Over a 15-day study period, TS-treated cattle achieved both superior appetite and lesion healing scores with more rapid reduction in dimensions of lesions than other cohorts, including a more rapid return to both eating and mobility, with earlier cessation of ptyalism and overt lameness. More recent observations include improved healing of decubitus ulcers occurring in recumbent cattle. Commencing in 2021, a series of trials have used the product for treating the oral lesions of lambs infected with Orf, demonstrating improved animal demeanour and reduced viral loads following treatment. Recently, in late 2023, a series of EHD-affected cows were treated with the product, following periods of up to 4 days of inappetence and ptyalism due to severe erosions of the buccal mucosa. Almost immediately following liberal application of the product to the oral mucosa, the affected cows commenced to eat and farmers expressed appreciation of the rapid clinical improvements in their animals.

Conclusions

These trials indicate that the product was very efficacious in blocking nociception from oral viral vesicular lesions, with almost instant relief of the pain usually sufficient for the animal to prehend food and drink. The findings confirmed that FMD, Orf and EHD are debilitating and painful disease with very negative animal welfare impacts that should be addressed. As farmers expressed their desire that the product be made available for use and financial modelling indicated that TS therapy imposed no additional financial burden on farmers, registration of the product in a range of countries is proceeding. As unnecessary use of antibiotics risks AMR, and residues in the food chain, it is suggested that TS offered a very viable non-antimicrobial approach to FMD, Orf and EHD therapy that is more welfare-appropriate and should be

promoted, particularly with the recent increased occurrence of FMD outbreaks in Asia (e.g. Indonesia) and EHD outbreaks in Europe.

Reference: Windsor, P.A. (2022) Role of Topical Anaesthesia in Pain Management of Farm Animals, a Changing Paradigm. Animals, 12,2459. https://doi.org/10.3390/ani12182459

1757 - Rational design of an effective ruminant sub-unit Q fever vaccine

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Objectives
The bacterium *Coxiella burnetii* can cause the disease Q-fever in a wide range of animal hosts. In ruminant livestock, infections generally lack clinical signs prior to the onset of adverse reproductive outcomes, including birth of weak offspring, abortion, and infertility. Ruminants, and in particular sheep and goats, are the main reservoir of infection for humans, where symptoms of Q fever range from acute flu-like symptoms to persistent focalised infections. Vaccines are considered the most effective control against *C. burnetti*. The only existing livestock vaccine Coxevac® (Ceva Santé animale, Libourne, France) is a killed bacterin vaccine based on the virulent phase I *C. burnetii* Nine-Mile strain, which has been licenced for use in goats and cattle since 2010, and in sheep since 2023. While Coxevac® is highly effective in reducing bacterial shedding and clinical disease, the vaccine commonly induces adverse reactions and is difficult to produce due to the requirement for manufacture at Biosafety level 3. The aim of this study was to identify *C. burnetii* candidate vaccine antigens and design an effective sub-unit vaccine for use in livestock.
Material and methods
High density peptide microarrays representing all sequenced *C. burnetii* open-reading frames (ORFs) were probed with sera generated from host species (bovine, goat, sheep, human) that were either previously infected with or were vaccinated against *C. burnetii* to identify seroreactive antigens. The ORFs of 15 antigens with the highest summed seroreactivity across all species were codon optimised for expression in *Escherichia coli*, cloned into the pET21 expression vector and expressed in ClearColi™ competent cells. Six vaccine candidate antigens were successfully purified and formulated into two vaccines, each containing three antigens plus Montanide™ ISA 61VG adjuvant. The protective effect of vaccine formulations was tested in a *C. burnetii* mouse challenge model measuring splenomegaly, using Coxevac® as a positive control. Sheep were also immunised with each of the six antigens individually and antibody responses evaluated to determine immunogenicity of the antigens in a relevant target species.
Results
A total of 493 seroreactive antigens were identified using high density peptide microarrays. The sub-cellular localisation of all antigens was determined and only those with outer membrane or extracellular loci were considered for vaccine development. From this group, 15 antigens with the highest summed seroreactivity across all species were successfully expressed in *E. coli.* From these antigens, six were selected based on purity and protein yield for further testing in mice. The six antigens were formulated into two subunit vaccines containing three antigens each plus Montanide™ ISA 61VG adjuvant and were used to immunise mice in two independent *C. burnetii* challenge trials. Both subunit vaccines significantly reduced splenomegaly in mice compared to unvaccinated controls, but to a lesser extent than that seen in the Coxevac® positive control group. Sheep immunised with each individual antigen formulate with either Montanide™ ISA 61VG or QuilA® adjuvant generated high titres of serum antibody to all six antigens.
Conclusions
Peptide microarray technology was successfully used to identify potential vaccine candidate antigens against *C. burnetii*. Two initial vaccine formulations showed efficacy in a mouse *C. burnetii* challenge model and all six antigens were immunogenic in sheep. This study provides proof-of-concept data for Q fever subunit vaccines based on recombinant proteins. However, the subunit vaccines tested in this study will require further optimisation in terms of adjuvant formulation and/or antigen selection prior to testing in ruminants.

1325 - Investigating Digital Dermatitis on dairy farms: exploring bacterial dynamics and mapping reservoirs

Author: Angelica Petersen Dias , Corienne Gammariello, Karin Orsel, Jeroen De Buck

Objectives

Digital Dermatitis (DD) is an infectious hoof disease with an uncertain etiology. Although multiple *Treponema* spp. are constantly present in lesions, other bacterial species, such as *Porphyromonas, Bacteroides, Fusobacterium*, and *Mycoplasma,* have also been consistently detected. While microbiome analysis has been informative about the core bacteria associated and potential reservoirs, absolute bacterial abundance for each of the species remains unknown, which is crucial for understanding disease pathogenesis. To address these gaps in knowledge, our research group developed a multiplex species-specific quantitative PCR (qPCR) targeting DD potential pathogens. We have also found swabs to be a reliable alternative to tissue biopsies when employing qPCR, allowing convenient multiple sampling from individuals. Thus, we conducted two studies to advance our understanding of DD microbiology. In Study 1 (S1; cross-sectional), we investigated potential reservoirs of DD-associated bacteria in environmental samples and various sites of dairy cow's body (lesions, skin, saliva, urine, and feces) from animals with or without DD in DD-free or DD-affected herds. In Study 2 (S2; longitudinal), we tracked bacterial load changes over time, before and during a DD episode, and investigated their roles in lesion initiation. We also aimed to determine whether the bacteria found in potential reservoirs identified in S1 are transient or persistent.

Material and methods

Both studies were conducted on commercial dairy farms in Alberta, Canada. In S1, we sampled 85 Holsteins dairy cows from five herds (three DD-affected, two DD-free). Swabs were collected from foot, hock, and udder skin (with lesions or not), as well as saliva, urine, feces, and environmental samples from free-stall alleyways and bedding material. In S2, we collected weekly samples from 53 Holsteins dairy cows in one DD-affected herd. Swabs were taken from both hind feet without DD lesions (M0 stage) and from the oral cavity (saliva) for at least 12 weeks, or until a DD lesion appeared. Cows were categorized into three groups: those that remained healthy (DD-), those that developed DD (DD+), and those that developed heel erosion (HE). Bacterial genomic DNA was extracted from samples and subjected to real-time qPCR targeting *Treponema phagedenis, Treponema medium, Treponema pedis, Porphyromonas levii, Fusobacterium necrophorum,* and *Bacteroides pyogenes*.

Results

In S1, DD-associated *Treponema* spp. were exclusively detected in DD-affected herds in DD-foot and skin lesions, healthy skin, saliva, and farm environment. In contrast, the other anaerobes were present in samples from both DD-free and affected herds. As expected, DD lesions had higher bacterial loads compared to healthy skin. None of the targeted species were detected in fecal samples, but the other anaerobes were present in urine. All targeted species were present in saliva, although in low quantities. In S2, out of 53 cows, 8 developed DD, while 31 remained healthy, and 14 developed HE. Analyses of S2 results are in the early stages as of the time of writing this abstract, and we anticipate completion by the 2024 conference.

Conclusions

For the first time, we quantitatively analyzed putative DD pathogens in various body sites of dairy cattle, including DD-free herds. We found no evidence that feces or urine were the primary sources of DD bacteria, but saliva and skin may have played a role. We concluded that DD-*Treponema* spp. in the environment likely originated from DD lesions rather than other sources. Our longitudinal study will be the first to detect species introduction and measure changes in bacterial load during lesion initiation, as well as their persistence in potential reservoirs. We anticipate an increase in *Treponema* spp. numbers before and during lesion onset, while the non-*Treponema* spp. anaerobes will increase only after lesion establishment.

1074 - Combining sampling techniques increases detection of carriers of Mycoplasma bovis

Author: Linde Gille, Julien Evrard, Laurent Delooz, Fabien Grégoire, Geert Vertenten

Objectives

Mycoplasma bovis' involvement in pneumonia, arthritis, mastitis, and other diseases as a primary pathogen has been well established in previous decades. Introduction of this bacterium into naïve herds inevitably results in important economic losses and reduced animal welfare. Given these losses, the inefficient therapy options due to its fastidiousness and innate and increasingly acquired antimicrobial resistance, and the lack of an effective protective vaccine, introduction of this bacterium needs to be avoided. Purchase of asymptomatic carrier animals is considered to be the major cause of introduction of *M. bovis*. While testing animals for the agent is, in theory, a foolproof way of avoiding the introduction; in practice identification of carrier animals is anything but straightforward.

No ideal testing strategy exists, due to *M. bovis'* tendency to shed intermittently at different sites combined with current suboptimal testing methods. Antibody ELISA testing allows for the identification of animals having been exposed to the bacterium beforehand, but previous research showed that only a small minority of these animals will become shedding carriers. Lastly, serology for *M. bovis* has been shown to have a poor sensitivity. Previous research even showed that bulk tank milk could be serologically negative but PCR positive for *M. bovis* when antibody carrying cows dropped below 30% of the herd. Real-time PCR on the other hand is a very sensitive technique, but bacterial DNA needs to be present in the samples. Currently, nasal swabs are being used most often as a quick, easy-to access sampling technique to test for carriage. However, given the recent studies showing a diversity in *M. bovis* carriage sites, from tonsils, to lung, to genital area, to the udder, this field study aimed to assess the relevance of combining different sampling sites to increase the possibility of detecting *M. bovis* carriers. Furthermore, in light of recent studies for other respiratory pathogens such as SARS-Cov-2 showing a significant increase in carriage detection when sampling both nasal openings, part of the nasal samples were performed in double to test the hypothesis that this would also increase *M. bovis* detection rate.

Material and methods

Six herds with active *M. bovis* circulation within the two months before the study were selected: 5 dairy herds, and one semen collection center. In total, 55 cows and 15 bulls were conveniently selected. Samples of these animals were taken bimonthly for a year. A total of 359 nasal swabs (NS), 348 swabs of the genital area (GS) (258 vaginal swabs (cow) + 90 sheath swabs (bull)), 258 milk samples (MS) and 87 semen samples (SS) were taken. Most of the NS were taken in a single nasal opening. On 76 occasions, nasal swabs were obtained from the 2 nostrils independently. A PCR was performed on each sample individually using LSI VetMAX Mycoplasma bovis® (Thermo Fisher Scientific).

Results

• 32 out of 70 animals tested at least one time positive on PCR during the follow up.

• Of the 55 cows, 36 never had a positive sample. Eighteen tested positive one time, on 1, 2 or all of their sample sites, while one tested positive at 2 different moments. In total, positive samples were: 8*NS, 6*GS, 1*MS, 2*NS+GS, 1*GS+MS and 2*NS+GS+MS.

• Amongst the 15 bulls, 13 had *M. bovis* detected on at least 1 sample, four of which were positive at 2 different moments. In total, bulls tested positive for: 9*NS, 4*GS and 4*NS+GS. Interestingly, none of the SS tested positive.

• From the samples taken separately from each nasal opening (n=76), 8 out of 13 positive animals only tested positive in a single nasal opening.

Conclusions

The detection of *M. bovis* carriers will be increased if animals are tested in parallel in different sites, at least the nasal opening and the genital opening should be sampled. Furthermore, 8 out of 13 (62%) positive animals were only positive in a single nasal opening. As such, it is highly recommended that both nasal openings are being swabbed when trying to identify *M. bovis* carriers.

1159 - Efficacy of a modified-live attenuated Mycoplasma bovis vaccine in 1 week old calves against a virulent Mycoplasma bovis challenge strain

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Objectives
Mycoplasma bovis is a causative agent in the Bovine Respiratory Disease complex, which is a multifactorial problem, influenced by environment, stress and several viral and bacterial pathogens. The purpose of the study was to determine the efficacy of a modified live attenuated *M. bovis* vaccine [Protivity] administered subcutaneously (SC) to one week old calves. Clinical and immunological parameters were used to evaluate the efficacy of the vaccine in an experimental challenge model using a heterologous virulent *M. bovis* strain.
Material and methods
Male and female Holstein calves acquired from a commercial dairy farm were included in the study. The study was conducted under the supervision of the Zoetis Institutional Animal Care and Use Committee (IACUC). All animals were colostrum deprived and seronegative as evidenced by ELISA and negative for *M. bovis* using enrichment culture of nasal swabs. Eighty-five animals were randomly assigned to one of three treatment groups. At 7-12 days of age, 40 calves (treatment group 2) were administered via the SC route with the vaccine, followed 21 days later with a second dose. Forty additional animals (treatment group 1) were included as control and were inoculated twice with Saline. Five calves were assigned to treatment group 3 and used as sentinels for lung lesion scores prior to challenge. Animals were experimentally challenged with a virulent *M. bovis* strain on day 32 and euthanized for lung lesion scoring on day 60. To assess post-challenge clinical signs such as nasal discharge, respiratory distress, and attitude, animals were observed daily from day 31 through day 60. Rectal temperatures were also collected. At study end, lungs were assessed for lesions and scored via a weighted scoring system, which allocates each of the lobes according to the relative weight of the lung lobes. The weighted lung lobe values were then summed across lobes to yield the percentage of total lung with lesions for each animal.
Results
Post-challenge observations showed higher frequencies of clinical signs in control animals compared to vaccinates. Overall, there was a significantly lower (P = 0.0005) mean number of days with abnormal clinical signs seen in the vaccinates (1.3 days) versus the controls (5.7 days). Mean rectal temperatures were significantly higher ($p<0.05$) in the control animals compared to the vaccinates. Pyrexia was significantly longer in the control animals (mean of 9.3 days), compared to vaccinates (mean of 2.9 days; P= 0.0009). The percentage of total lung with lesions (least square means) were 19% in the control group compared to a significantly lower 5% in the vaccinated group (P=0.0001). The mitigated fraction estimate which measures the relative increase in the probability that a vaccinate's lung lesions will be less severe than a non-vaccinate's lung lesions was 0.66 (95% CI: 0.37, 0.90), which was further supported by the 5-number summary comparing percentage of total lung with lesions between control and vaccinates. Serological responses were analysed using an ELISA to detect anti-*M. bovis* antibodies. Statistical analysis of the obtained titres showed a significantly higher mean titre of *M. bovis* antibodies in vaccinates compared to controls on day 31 (pre-challenge) and day 60 (study end).
Conclusions
The findings of the present study confirm the efficacy of a modified live attenuated *M. bovis* vaccine [Protivity] in young calves against experimental challenge, through the reduction in lung lesions, respiratory clinical signs, and duration of pyrexia after challenge associated with an induction of an immunological response.

1448 - Comprehensive whole genomic analysis of Escherichia coli and Salmonella spp. isolates in neonatal diarrhea among calves from Latin American Industrial Dairy Farms

Author: Luis León Paredes, Diego Flores, Sofía Zamudio, Karen Mujica, Pabla Lara, Nelson Garrido, Rodrigo Norambuena, Victoria Rojas-Martínez, Andrea Sabag, María Jesús Serrano, Michael Pino, Paola Mora, Valeria Salgado, Daniel Tichy, Pamela Camejo, Néstor Roca, Pablo Cifuentes, Soledad Ulloa, Nicolás Cifuentes, Geert Vertenten, José García, Vanessa Masson, Guillermo Gargantini, Marco Gallardo, Felipe De La Fuente

Objectives

- Characterize the serotype diversity in *E. coli* and *Salmonella* spp. present in calves with neonatal diarrhea in industrial dairy farms of LATAM.
- Identify virulence genes linked to distinct pathotypes and serotypes within *E. coli* and *Salmonella* spp. which potentially cause neonatal diarrhea in calves of industrial dairy farms in LATAM.
- Analyze the prevalence of antibiotic and disinfectant resistance genes within isolates of *E. coli* and *Salmonella* spp. isolates. from calves with neonatal diarrhea in industrial dairy farms in LATAM.

Material and methods

More than 90 dairy farms from productive regions of Argentina, Brazil, Chile, and Mexico were enrolled for the study. Rectal swabs were collected from calves up to 3-weeks old, with fecal consistency score 2 or 3 (n=722). Swabs were enriched, and the presence of *Escherichia coli* pathotypes was determined by qPCR. In parallel, enrichments were inoculated in *Salmonella* plus CHROMagar to isolate *Salmonella* spp. Colonies were reisolated three consecutive times and subsequently tested by qPCR.
DNA from *Salmonella spp.* and *Escherichia coli* strains was extracted, libraries prepared, and sequencing performed through Illumina platform. Genomic sequences were then analyzed using a custom workflow pipeline developed at PhageLab SpA. The resulting genomes were subjected to gene annotation, identification of potential virulence factors, and antibiotic/disinfectants resistance genes.

Results

Microbiological positivity of samples for *Escherichia coli* was 5.63%, 7.04%, and 11.27%, whereas for *Salmonella* it was 3.13%, 1.51%, and 8.45% for Argentina, Brazil, and Chile, respectively. To date, a collection of 365 isolates has been obtained from the farms of Argentina (n=132), Brazil (n=113), and Chile (n=119). Of these, 83 isolates were positive for ETEC, STEC, EPEC or EHEC markers, and 14 isolates were positive for the *Salmonella* marker. From the group of *E. coli*, 51 isolates were preliminarily identified as STEC (61.4%), and 21 isolates were identified as EPEC (25.3%). 47% of STEC isolates were obtained from Brazilian farms and 39% from Chilean farms, whereas EPEC isolates came mainly from Chile (57%) and Brazil (33%).

A total of 87 isolates (14 *Salmonella* and 73 for *Escherichia*) were evaluated by whole genome sequencing. Genomic analysis for *Salmonella* revealed that Typhimurium (64.29%), Dublin (14.29%), and Cerro (14.29%) were the most prevalent serovars. Isolates have predicted resistance to several antibiotic families, being the most prevalent aminoglycosides (100%), fluoroquinolones (64%), and tetracyclines (42.8%). Moreover, two Typhimurium isolates from Argentina were predicted to contain gene resistance to all antibiotic families evaluated.

Of the 73 *Escherichia coli* isolates, only 60 passed the bioinformatic pipeline quality metrics. These 60 isolates showed a high diversity in serotypes, being the most frequent O103:H2 (20%), O111:H8 (10%), and O153:H2 (10%). Interestingly, all isolates were predicted to be resistant to

several families of antibiotics including aminoglycosides, fluoroquinolones, macrolides, tetracycline, phenicol, and rifamycin.

Conclusions

Escherichia coli pathotypes are distributed in all farms evaluated. Around 62% of *E. coli* isolates are STEC and 25% STEC. Moreover, EPEC and STEC were found mainly in Brazil and Chile. These pathotypes contain group-specific virulence genes including shiga toxins, enterotoxins, and fimbriae among others. These isolates exhibit a heightened virulence potential, posing a significant threat to host cells and facilitating efficient colonization. On the other hand, the identification of antibiotic-resistant *Salmonella enterica* and *Escherichia coli* in veterinary contexts underscores a pressing public health issue. The coexistence of these pathogens, resistant to several used antibiotics, emphasizes the need for vigilant surveillance and judicious antibiotic use. Notably, the diverse serotypes and serovars observed for *E. coli* and *Salmonella*, respectively, further complicate the landscape of antimicrobial resistance. Addressing this challenge requires a comprehensive approach involving antimicrobial use, enhanced biosecurity, and ongoing research to develop safe and effective alternative strategies for infection (like bacteriophages), thereby safeguarding both animal and human health.

On November 22nd, the processing of the last samples from Mexico (296 samples) began and the results will be available on January 22nd. On this date, all results will be updated to complete the discussion of this project.

1461 - Effect of Salmonella Dublin latent-carrier dry cow vaccination on bacterial shedding and intrauterine transmission

Author: Angel Abuelo , Rafael Castro-Vargas, Faith Cullens-Nobis, Jennifer Roberts

Objectives

Salmonella Dublin latent-carrier cows represent a high risk for the transmission of infection to newborn calves via intra-uterine transmission and shedding of bacteria in feces and colostrum at calving. Vaccination of these latent-carriers dams during late gestation boosts the immunity against *S.* Dublin. This could reduce the activation of the dormant bacterium during the periparturient immune dysfunction period, thereby reducing the risk of early-life infection in the offspring. Thus, the objective of this study was to evaluate the extent to which vaccinating *S.* Dublin latent-carrier cows at dry-off with a commercial live bacterial vaccine (Entervene-D, Boehringer Ingelheim) reduces bacterial shedding at calving and intrauterine infection to calves.

Material and methods

To identify latent-carriers, we screened 1,084 cows in 4 Michigan commercial dairy farms with a history of *S.* Dublin. Cows were defined as latent-carriers when they showed three consecutive positive milk antibody ELISA tests conducted every two months. Subsequently, 148 latent-carriers were randomly allocated to the vaccine or control group. Vaccine cows received the commercial vaccine s.c. at dry-off and a booster two weeks later. Control cows received saline s.c. at the same times. At calving, we collected fecal and colostrum samples from the dam and a pre-colostral serum sample from the calf. Bacterial shedding was evaluated in feces and colostrum both qualitatively (Yes/No) and quantitatively through the bacterial enrichment culture method (ISO 6579-1:2017) and qPCR quantification of gene *vagC* copy numbers, respectively. Intrauterine transmission was defined when a calf was positive for serum antibody ELISA at birth. Results were evaluated via logistic regression for qualitative shedding and intrauterine transmission. A t-test was used to compare the number of *S.* Dublin copies estimated via qPCR.

Results

Vaccination decreased the likelihood of calves being born with *S.* Dublin antibodies (Relative Risk [95%CI]) = 0.19 [0.04 – 0.84]). However, no *S.* Dublin-positive isolates were identified through either bacteriological culture or qPCR in feces or colostrum.

Conclusions

Vaccination of *S.* Dublin latent-carrier cows at dry-off reduced intrauterine transmission to calves. This strategy could contribute to decreasing the transmission of *S.* Dublin in dairy farms. Additionally, the absence of *S.* Dublin positive fecal and colostrum samples warrants further evaluation of the traditional methods for identification of latent-carriers or *S.* Dublin isolation, as well as the role of latent-carriers in infecting newborn calves in the maternity area at birth.

1029 - Ovine Clostridium septicum epidermal necrolysis dermatitis: A herd report

Author: Karima Al Salihi Al Salihi, Entissar Mansour Abdul Rasool

Objectives: This study intends to report severe epidermal necrolysis dermatitis in a sheep herd raised in the Desert of Samawa in November 2021. In addition, to isolate causative agents associated with the current epidermal necrolysis dermatitis using bacteriological, biochemical tests, and polymerase chain reaction tests (PCR). Moreover, to investigate the histopathological changes.

Case herd description: Unpredicted, sudden death appeared in several Awassi sheep in a herd raised in the Desert of Samawa in November 2021. Most affected sheep had distinguished elevated purple lesions comprising the buttock, neck, ears, and ventral trunk. Moreover, the affected animals suffered from fever, rapid respiration, and heart pulsation and presented in a recumbent position with deteriorated and terminating in death. Upon clinical examination, layers of skin were sloughed and fallen, leaving extensive naked uncovered areas. Samples were collected from the margins of skin lesions and underlying skeletal muscles.

Materials and methods: The samples were divided into two parts. The first part was sent for microbiological investigation, and direct smears were stained with Gram stain. Moreover, the samples were cultured anaerobically on 10% sheep blood agar at 37 oC for 72 hours in anaerobic conditions, and biochemical tests characterized the isolated suspected bacteria. The second part was sent for routine histopathological examination. Isolated bacteria were amplified for 270 bp DNA fragments, and a primer set was used, according to Takeuchi et al. (1997). The 5´-AAT TCA GTG TGC GGC AGT AG-3' positioned on the hemolysin gene from pb 611-631 was used as a forward primer. While the reverse primer was 5'- CCT GCC CCA ACT TCT CTT TT-3' complimentary to 861-880 of hemolysin gene. The following formula was used to determine the Tm= 4(G+C) + 2(A+T)

Results

Significant lesions were seen in the skin biopsy. A necrotic dermis (infarcted) and sloughed epidermis. The dermis and subcutis were extended by edema, infiltration of inflammatory cells, predominantly neutrophils, and fibrin accumulation. Additionally, bundles of degenerated myocytes surrounded by fibrin and neutrophils were also seen. Numerous long bacilli compatible with clostridium spp. were visible among the inflammatory debris. The bacteriological examination of the cultured samples revealed a spore-bearing, gram-positive anaerobic bacilli characterized as Clostridium. On blood agar, the growing colonies were 3-4 mm in diameter, semi-translucent greyish, slightly raised with irregular margins, and a clear zone area of beta hemolysis. Swarming surrounded each colony. All isolated colonies were liquified gelatine and clotted the milk-producing acid but did not produce hydrogen sulfide and indole. The colonization was negative for urease and lecithinase. On egg yolk agar, the colonies did not produce lipase but nitrate reductase in nitrate broth. All isolated bacteria fermented glucose, maltose, and salicin, but not sucrose, lactose, mannitol, and esculin, identified as Clostridium septicum accordingly. The isolated bacteria showed a 270-bp band on agarose gel electrophoresis, which proposes a conserved sequence for the lethal toxin in Cl. septicum isolates.

In conclusion, this study reported an unusual presentation of ovine epidermal necrolysis dermatitis called Clostridium septicum dermatitis according to histopathological changes, bacterial culture, and polymerase chain reaction tests as an important confirmation test for the diagnosis.

Keywords: Clostridium septicum, dermatitis, epidermal necrolysis, sheep.

Reference

Takeuchi, Sh., Hashizome, N., Kinoshtta, T., Kaidoh, T. and Tamurai, Y. (1997). Detection of Clostridium septicum hemolysin gene by polymerase chain reaction. Journal of Veterinary Medicine Science 59(9): 853-855.

1420 - Dichelobacter nodosus from footrot outbreaks in Uruguayan sheep herds

Author: Ludmila Slimovich Eguiño, Ana Crescionini, Sofía Salada, Sofía Fernandez_Ciganda, Martín Fraga

Objectives

Foot-rot is the most important foot disease in sheeps and is caused by Dichelobacter nodosus. Affected animals lose well-being and productivity. Dichelobacter nodosus can be serogrouped by means of the fimbrial protein codified by FimA gene. So far, ten serogroups have been described. To control the disease vaccines against fimbrial proteins have been designed. In Uruguay, control approaches include the stock inspection followed by the elimination of the chronic or irrecoverable animals. Monovalent, or bivalent vaccines, with the specific serogroups responsible for the disease in a said flock, have shown great potential for prevention, cure, and eradication in sheep sectors worldwide. In our country there is little updated information regarding the active serogroups of D. nodosus, and this is very important for disease control strategies through serogroup-specific vaccinations. The objectives of this work were to detect D. nodosus by PCR and determine the serogroups circulating in Uruguayan herds, our country, to isolate and characterize native D. nodosus, and compare the FimA gene sequences with sequences in databases.

Material and methods

An opportunity sampling was performed in 37 herds with previous diagnosis of Foot-rot. The ovines were clinically diagnosed by examining the hoof. In each flock, samples were taken from between 4 and 24 animals by swabbing the affected area (450 animals). When it was possible, samples were spreaded onto hoof agar for isolation. Total DNA was extracted from swabs and isolates and the presence of D. nodosus was detected by means of a specific PCR. In the positive samples, circulating serogroups were determined by multiplex-PCR. The FimA gene (fimbrial protein gene) was amplified and sequenced and further compared with a previous database.

Results

Thirtyseven flocks were positive to D. nodosus. Taking into account the animals, 68.2% were positive. Serogroups A, B, C, D, E, F, G, H and I were detected, with E being the most frequently found (53,1%) and B (32,9%). Between 1 and 7 serogroups were diagnosed per establishment, and more than 70% of the farms had only one or two serogroups at the same time. It was possible to analyze 15 FimA sequences, 7 of them were isolates from 4 herds, observing that three of the sequences obtained did not clustered with any of the sequences of the same serogroup from other regions.

Conclusions

There is no previous report of serogroups G, H and I in Uruguay. Previous reports from 2011 using microagglutination described that F as the most abundant serogroup followed by E and A. In our work serogroup E is the most frequently detected serogroup followed by B. Serogroup M was not tested because there is note a specific PCR for it. All the isolates were serogroup A B, C, D or E, no F G H or I isolates were achieved. Three FimA sequences were not clustered with the sequences of the same serogroup from other regions, these sequences were from serogroups B and E that are very variable worldwide. This fact can interfere in the efficiency of vaccines and highlights the importance of designing specific vaccines.

The fact that most of the flocks have only one or two serogroups encourage to continuing the determination of serogroups as a starting point for different disease control programs with serogroup-specific vaccination.

1769 - Bovine Immunodeficiency Virus seroprevalence in Great Britain and immunophenotyping in animals co-infected with Mycobacterium bovis

Author: Neil Watt, Sarah Eikenbusch, Linda Scobie, Anna Raper, Jayne Hope

Objectives

1. To assess whether co-infections with BIV and M bovis altered immune cell populations and whether this may affect the diagnosis of bTB.

Material and methods

Blood samples from 800 cows on 50 farms in GB are were subject to ELISA for BIV using a peptide from the gp41 transmembrane glycoprotein. 30 samples were obtained from a 500-cow dairy herd with on-going Bovine Tuberculosis (BTB) for analysis of immune cells. The BTB status of the cows was determined using the Enferplex Bo vine TB antibody test and and BIV status was determined using PCR. Samples were grouped into bTB- BIV-, bTB- BIV+, bTB+ BIV-, and coinfected bTB+ BIV+.

Peripheral blood mononuclear cells (PBMCs) were prepared by standard centrifugation methods, washed and reconstituted in freezing medium for storage at -155^{o}C. Recovered samples were subject to flow cytometric analysis using markers for CDs 2, 3, 4, 8, 11b, 14, 16, 21, 172a, 163, 205, WC1, NKp46 and MHC2. Samples were gated for cells, single cells, then live cells and at least 50,000 gate capture events were collected and at least 100,000 events were collected from the natural killer (NK) cell staining panel. Statistical analysis of data was performed using GraphPad Prism software. Dunnet's multiple comparisons test was used for all data sets.

Results

Dendritic cells A significantly (p=0.005) lower percentage of DCs was observed in bTB+ BIV- cattle compared to uninfected cattle. In addition, bTB- BIV+ cattle had a significantly lower (p=0.02) CD205+ MHCII+ population than uninfected cattle.

T cells Cattle that were bTB+ BIV- had a significantly higher percentage (p=0.03) of CD4+ cells than uninfected cattle. In addition, cattle co-infected bTB+ BIV+ had a significantly lower CD8+ population (p=0.04) when compared to bTB+ BIV- cattle. No significant difference in CD4+ CD8+ cells, CD8+ cells, or CD8- WC1+ cells in infected cattle were observed when compared to uninfected cattle

B cells B CD11b+ CD21+ B cells were present at a significantly smaller percentage (p=0.0095) in cows that were bTB+ BIV- compared to uninfected cattle. CD11b+ CD21- cells were also tested but no significant difference was observed between infected and uninfected cattle

NK cells, NK T cells and monocytes No significant differences were noted between infected and uninfected cattle.

Conclusions

1. TB+ BIV+ had a smaller CD8+ population compared to TB+ BIV- cows. It is possible that a reduction in CD8+ T cells could result in lower IFN-γ production, affecting the ability of the IFN-γ diagnostic test to correctly identify TB infected animals when used in conjunction with the SICCT test.

2. The proportion of CD4+ T lymphocytes was increased in TB+ BIV- cattle and relatively increased in TB- BIV+ cattle.

3. Fewer CD21+ CD11b+ B cells were identified in TB+ BIV- cattle compared to uninfected cows. This may indicate that B lymphocytes are being activated and then recruited to the site of infection. However, cattle TB+ BIV+ did not have a similar reduction in the circulating

B cell population when compared to uninfected cattle, which suggest that a concurrent infection with BIV may affect B cell function during a bTB infection.

4. The proportion of DCs was significantly decreased population in both TB- BIV+ and TB+ BIV- infected cattle when compared to uninfected cattle. This may indicate recruitment of these cells to sites of infection. In contrast, cattle that were co-infected (TB+ BIV+) did not show a significantly lower DC population in the blood. This suggests there may be difference in the ability of the DCs of co-infected cattle to properly activate, migrate, and present TB antigen.

5. Currently used diagnostic tests for *M. bovis* in GB require an active immune response for accurate detection of bTB infected cattle, further studies of the functionality of cell populations of TB+ BIV+ cattle compared to uninfected cattle will be important. It has been shown that PMBCs in cattle infected with *M. bovis* are prevented from proliferating, but *M. bovis* infection does not affect the cytokine response. A cytokine assay would be useful to characterize the difference in immune response, would provide more insight into the efficacy of the immune response of the coinfected cattle, and could further elucidate the effect BIV has on diagnostic tests for bTB.

1676 - In vitro antimicrobial resistance of bacteria isolated from bovine clinical mastitis in Germany

Author: Luis Leon, Christian Fidelak, Marcus Klawitter, Ulrich Löschner, Ansgar Busch, Thomas Breuer, Torsten Steppin, Maria Elena Vergara Hernandez

Objectives

The goal of this study was to analyze the clinical mastitis pathogens resistance to routinely used antibiotics by analyzing samples collected across different regions in Germany.

Material and Methods

Quarter milk samples of cows with clinical mastitis were analyzed at a milk quality laboratory (bovicare GmbH, Germany). The samples were processed for bacteriology, SCC (Fossomatic, FOSS Analitical) and resistance (agar diffusion test) against the most frequently intramammary used antibiotics / antibiotic fixed combinations: amoxicillin and clavulanic acid (AMC), cephapirin (CEPA), cephalexin and kanamycin (CKN) and penicillin (P).

The samples were submitted by local veterinarians during September 2015 and November 2023. The samples were examined following NMC guidelines. Resistance was determined using agar diffusion method, evaluation of the inhibition-zone-results (S: susceptible, I: intermediate, R: resistant) was done using literature and companies' recommendations. The resistance results were statistically analyzed (IBM-SPSS; version 26.0) using Cochran-Q-test (S+I versus R) followed by Bonferroni-Dunn post hoc test. The Kruskall-Wallis-test with the Bonferroni-Dunn correction was used for analysis of the region's effects. Differences consider statistically significant when $P<0.05$.

Results

A total of 5.011 quarter bacteriological positive samples were tested for resistance. The most frequent pathogen was *Streptococcus uberis* (35%), followed by *E. coli* (13,3%), *Staphylococcus aureus* (12,8%), Coagulase negative *Staphylococcus (CNS)* (10,7%), coliforms (*Serratia spp., Klebsiella spp*.) 8,2%, *Streptococcus dysgalactiae* (8,1%) and *Enterococcus spp.* (6,4%). This pathogen distribution is related in the literature (Schmenger and Krömker, 2020). The results showed a significant difference of the in vitro susceptibility between the antibiotics tested (p=0,000). For *S. uberis* the distribution of resistant-isolates was: CKN (8%), P (7%), CEPA (6%), AMC (1%), The frequency of resistant *E. coli* isolates was: P (100%), CEPA (72%), CKN (34%), AMC (5%). The resistance situation for the *S. aureus*-Isolates was: P (26%), CKN (13%), AMC (5%) and CEPA (3%) and no differences across the regions were found. The frequency of resistant *CNS* isolates was: P (48%), CEPA (4%), CKN (4%), AMC (4%).

For coliforms the percentage of resistant isolates was: P (100%), CEPA (54%), CKN (27%), AMC (20%). *S. dysgalactiae* showed the resistant distribution: CEPA (4%), CKN (4%), AMC P (3%), (1%). The *Enterococcus spp*. showed the resistant situation: CKN (71%), CEPA (57%), P (14%), AMC (3%).

Conclusion

Large number of isolates showed resistance against P, CEPA and CKN confirming the hypothesis that some gram positive and gram-negative mastitis isolates are not susceptible to often used antibiotics. AMC shows an activity against the most important gram-positive and gram-negative pathogens. Similar results were published in Germany and other countries (Zieger et al. 2014, Leon et al. 2015, Leon et al. 2020, Bolte et al. 2020, Leon et al. 2022).

1552 - Field study comparing two intramammary antibiotics for the treatment of bovine clinical mastitis

Author: Luis Leon, Christian Fidelak, Marcus Klawitter, Thomas Breuer, Ansgar Busch, Torsten Steppin, Ulrich Löschner, Maria Elena Vergara Hernandez

Objectives

The treatment of clinical mastitis with critically important antimicrobials like cefquinome (cephalosporin 4th generation) is common in Europe. Since years, the use of this kind of antibiotics is strongly discussed by vets, dairy plants, retail food sector, consumers, and legislators. In Germany, like in other European countries, the administration of high critical antibiotics (cephalosporin 3rd and 4th generation, fluoroquinolones) required a susceptibility test. This means cost of producers and a new orientation for veterinarians looking for alternatives with broad-spectrum activity. The purpose of this field study was to compare the effectivity of two intramammary products (Cobactan® LC, MSD, NL and Synulox® LC Plus, Zoetis USA) on the clinical and bacteriological cure of spontaneous mild clinical mastitis.

Materials and methods

The field stugy took place in a commercial dairy farm in East Germany with 523 Holstein-Friesian dairy cows with Ø 10.243 kg lactation (fat 4,15%; 3,41% protein). After the appearance of a clinical mastitis at one quarter, the cows were randomized using the parameters “days in milk (DIM)” and “number of lactations” in the two treatment groups. A i.mm. syringe of Synulox® LC Plus contains 200 mg of the aminopenicillin amoxicillin, 50 mg clavulanic acid as potent inhibitor of many beta-lactamases and 10 mg prednisolone (anti-inflammatory corticosteroid). Cobactan® LC contains 75 mg cefquinome as broad-spectrum antibiotic. The antibiotics show similar in vitro susceptibility against mastitis pathogens (Steiner and Swinkels, 2017).

The treatments were applied according to the SPCs (one syringe into the teat of the infected quarter every 12 hours after each of three successive milkings). Only cows with one udder quarter with mild clinical mastitis (mastitis grade I, meaning thatchanges in milk such clots and flakes, watery appearance, etc.) were chosen to participate.

In the hours 0, 3, 6, 12, 24 and 36 each udder quarter was clinically assessed by the farmer (CMT – California mastitis test, palpation, and visual assessment of the milk). On day 7 an additional CMT was performed.

On days 0, 14 and 21 after first treatment milk samples were taken for visual examination and analysis. All milk samples were investigated cyto-bacteriologically according to the guidelines of the German Veterinary Medical Association (DVG, 2009) at the milk quality laboratory *bovicare GmbH* in Bernau (Germany). With the samples which were bacteriological positive, a susceptibility test (agar diffusion method) was carried out.

Results

In total 233 quarters with a mastitis were used for the statistical analysis (IBM SPSS Statistics 28). 114 (48,9%) were treated with “amoxicillin + clavulanic acid + prednisolone” and 119 (51,1%) with “cefquinome”.

The quarters treated with “amoxicillin + clavulanic acid + prednisolone” were from cows of with a geometrical mean of lactations of 3,15 +/- 0,13; the “cefquinome”-group shows a mean of lactations of 3,29 +/- 0,13 (p=0.938, ANOVA). The “amoxicillin + clavulanic acid + prednisolone”-group had a geometrical mean of 146 +/- 9 DMI, the group “cefquinome” 138 +/-9 (p=0,866, ANOVA).

The clinic signs (palpation, visual assessment of the secretion) decreased clearly across the time (hours 0, 3, 6, 12, 24 and 36 after the first treatment), none of the clinical parameters showed a significant difference between both treatments at any of the time points (Chi-squared-test across all time points p>0,05). The CMT (hours 0, 3, 6, 12, 24, 36 and day 7 after first treatment),

decreased in both groups in a very similar form (Chi-squared-test across all time points was p>0,05)

Conclusion

This study shows that compared with a high critical antibiotic (cefquinome), classified as category B ("restricted use") by the European Medicines Agency (EMA), the local use of a fixed combination of amoxicillin, clavulanic acid and prednisolone offers similar results for the clinical parameters and the bacteriological cure of clinical mastitis cases.

References

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Steiner and Swinkels (2017). Antibiotical susceptibility of pathogens isolated from mastitis cases in Germany. European Buiatrics Forum, Bilbao 4-6 Oct 2017. Poster ABSEBF00043

1021 - Main bovine respiratory infectious agents identified on stethoscopes and boots from 12 rural veterinary clinics.

Author: Thibault Jozan, Camille Levesque, Geert Vertenten

Objectives

Relapses of respiratory episodes in the same herd can be explained by the possible infection of non-immune animals by persistent environmental agents or re-excretion of pathogens by carrier animals during stress or immunodepression. Particular situations are the airborne transmission of agents between farms, the introduction of animals or through infected vectors (material and humans).

The objective of the study was to investigate the contamination of rural veterinary stethoscopes and boots with the main infectious agents of the bovine respiratory complex.

Material and methods

- 12 veterinary clinics have been included in the study in December 2022.
- Samples in each veterinary practice :
    - 2 - 4 pairs of boots and 2 - 4 stethoscopes were individually swabbed (dry swabs) by veterinarians over the weekend of rural activity.
    - Swabs were shipped to the analytic lab via cooled transport within 24 hours of sampling.
- Analysis at the lab (LABOCEA 35, France) :
    - For each of the veterinary practice, 1 pooled PCR was performed on the stethoscope swab samples and 1 pooled PCR on the swabs performed on the pairs of boots
    - Extraction was performed by the kit ID GeneTM Mag Universal (IDvet), following by Real-time PCR multiplex* analysis with VetMAXTM Ruminant Respiratory Screening Kit (Thermofischer) : experimental use in the absence of validation data for the tested sample types.

*7 BRD agents targeted : Bovine Respiratory Syncytial Virus (BRSV), Bovine coronavirus (BCoV), Para-influenza 3 (PI3), *Mycoplasma bovis* (Mb), *Mannheimia haemolytica* (Mh), *Histophilus somni* (Hs), *Pasteurella multocida* (Pm).

Results

Stethoscopes: 75% of the veterinary practices with at least 1 infectious agent identified by PCR.

Pairs of boots: 50% of the veterinary practices with at least 1 infectious agent identified by PCR.

The most frequently detected viral pathogen was BCoV, found on stethoscopes of a quarter of the veterinary practices and the boots of one veterinary practice. BRSV was found on the stethoscopes of 1 veterinary practice of the twelves sampled.

The most frequently detected bacterial pathogen was *Pasteurella multocida* (on stethoscopes of 8 veterinary practices and on boots of 3 veterinary practices).

Between 5 and 46 cattle farms (average: 26) have been visited by the veterinary practices during 72 hours before sampling.

In addition, 100% of pairs of boots and 40% of stethoscopes were disinfected during the 30 days before the sampling (1 to 14 days prior sampling for pairs of boots; 10 to 20 days prior sampling for stethoscopes)

The potential for infection was not assessed in this study. Oma et al. 2018 showed that BCoV and BRSV could remain infectious on this type of medium (at least 24h for BCoV).

Conclusions

A high contamination of BRD agents (genetic material) can be identified by PCR on pools of swabs from stethoscopes and pairs of boots. The risk of indirect transmission of respiratory agents by material and human vectors should be integrated in farm biosecurity.

1240 - Characterization of the lower respiratory tract virome in dairy calves with and without clinical respiratory disease signs

Author: Kerli Mõtus , Elisabeth Dorbek-Kolin, Marina Loch, Rohish Kaura, Dagni-Alice Viidu, Tarja Sironen, Ilja Weinstein, Toomas Orro

Objectives

Bovine respiratory disease (BRD) constitutes a major challenge in the dairy industry impairing cattle health, performance, and welfare, and it creates the need to use antimicrobials and induces economic loss. The use of next-generation sequencing technologies has allowed the genetic characterization of viral genomes in animal and human respiratory tract. Previous metagenomic studies have discovered the known and novel viruses associated with calves´ clinical respiratory disease. Respiratory viruses may hold the triggering role for invasion of secondary microbial communities and development of possibly fatal bronchopneumonia. By using the unbiased metagenomic approach, we aimed to characterize the respiratory virome of lower respiratory tract (LRT) samples collected from dairy calves presenting clinical BRD symptoms and healthy location-matched controls.

Material and methods

Between September 2021 and March 2022, twenty large-scale dairy herds (including more than 400 cows) were visited. Presence of the following clinical signs were recorded for each pre-weaned calf: presence of nasal and ocular discharge, spontaneous cough, respiratory rate, rectal temperature, navel inflammation, diarrhoea, and depression. In each farm, five (six in one farm) calves suspected to have LRT disease based on clinical signs and no history of previous respiratory disease nor any vaccinations ≤14 days before farm visit were sampled (called “diseased”). Also, five calves not suspected to have LRT disease (“healthy”) were sampled, resulting in a total sample size of 201 calves. Tracheobronchial lavage (TBL) samples were collected from all calves.

To clarify herd status for the main respiratory pathogens, bulk tank milk (BTM) and serum samples of ten randomly chosen heifers were collected. Samples were tested for the presence of bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV) and bovine respiratory syncytial virus (BRSV) antibodies if not vaccinated with non-marker vaccines.

TBL samples were stored in – 80°C until the extraction of viral nucleic acids using Qiagen Viral RNA mini kit (ref no: 52904). Samples were analysed at farm-level in two pools - one consisting of TBL samples of diseased calves and the other including TBL samples of healthy calves. In total, 40 TBL pools were sequenced using an Illumina MiSeq platform. An in-house analysis pipeline was used to process the data.

Viruses with at least 20 reads in pooled samples were included in the analysis. Pool-level frequency of different viral species were compared by using chi-square test.

Results

Out of 40 TBL pools, 9 different viral species were identified (with ≥20 reads). Severe acute respiratory syndrome-related coronavirus was the most often identified virus (n = 29) but was equally represented in the pools of healthy (n = 14) and diseased calves (n = 15). Other most frequently identified viruses were bovine rhinitis B virus (n = 16), betacoronavirus 1 (n = 16), ungulate copiparvovirus 1 (n = 14) and bovine mastadenovirus B (n = 6). For most identified viruses, there was no significant difference in the number of pools including healthy and diseased calves. However, the ungulate copiparvovirus 1 was significanlty more prevalent in pools sampled from diseased calves (10 diseased and 4 healthy calves´ pools, p = 0.047). Also, 96.4% of all ungulate copiparvovirus 1 reads were from pools sampled from diseased calves.

Based on BTM and heifer serological testing, 60% of the study herds were positive to BHV-1. In total, 33% and 100% of non-vaccinated herds were positive for BVDV and BRSV, respectively. These viruses were not identified in TBL pools.

Conclusions

The present study revealed the complexity of the virome present in the LRT of pre-weaned dairy calves reared in large-scale Estonian dairy herds. Several viruses that are not targeted in routine diagnostic testing nor included in commercial vaccines were identified. Pooled calf virome profiles were somewhat different in diseased and healthy calves. Still, the prevalence and pathogenicity of these emerging viruses should be further determined on animal level. Established viral BRD aetiological agents (BHV-1, BVDV and BRSV) were not detected in calf TBL samples despite their high prevalence among the study herds. The current work demonstrates that metagenomic sequencing is an unbiased method for detecting viruses in the calves´ respiratory tract.

This work was supported by an Estonian Research Council grant (PSG268) and base funding of the Estonian University of Life Sciences (P220165). The authors express their gratitude to all participating farms.

1637 - Effect of tulathromycin metaphylaxis on the nasopharyngeal microbiome, resistome, and Mannheimia haemolytica isolation in cattle

Author: Amelia Woolums

Objectives
This study aimed to determine the effect of tulathromycin metaphylaxis (mass medication for control of bovine respiratory disease (BRD])) and subsequent BRD treatment on antimicrobial resistance (AMR) in *MH* isolated from the nasopharynx, the respiratory microbiome and resistome, and the *MH* community in beef cattle.
Material and methods
Crossbred beef heifers (n=331, mean weight=232, SD= 17.8 kg) at high risk for bovine respiratory disease (BRD) were randomly assigned to receive tulathromycin (META, n=167) or not (NO META, n=164) at arrival. Cattle were evaluated daily and treated for BRD based on a pre-established clinical scoring system; META cattle were not eligible for BRD treatment before day 8 after arrival. Nasopharyngeal swabs (NPS) were collected for DNA extraction and *MH* isolation, antimicrobial susceptibility testing and whole genome sequencing at arrival and 3 weeks later (WK3). DNA extracted from NPS were pooled based on metaphylaxis administration and treatment for BRD, resulting in 4 treatment groups (TxGroups)—1) META-BRD, 2) META-NO BRD, 3) NO META-BRD, and 4) NO META-BRD. DNA pools underwent 16S rRNA amplicon sequencing, target-enriched antimicrobial resistance gene (ARG) and *MH* gene sequencing, and qPCR for *MH*. NPS were collected at arrival and 3 weeks later (WK3). Multidrug resistance (MDR) was defined as phenotypic resistance to 3 or more antimicrobial classes.
Results
META cattle had higher odds of isolation of MDR *MH* at WK3 than NO META cattle (OR (95% CI)=13.08 (5-30.9), *P*<0.0001). There was no difference in risk of isolation of any *MH* (resistant or susceptible) between META and NO META groups at any timepoint. Animals in the NO META group had 3 times higher odds of being treated for BRD by WK3 (OR (95% CI)=3.07 (1.70-5.52), *P*=0.0002). Antimicrobial resistance genes found within MH were associated with integrative conjugative element (ICE) genes. There was no effect of tulathromycin on richness or diversity of the microbiome on WK3. However, Shannon's diversity index decreased at WK3 compared to arrival, and there was a difference in microbial community structure in all TxGroups at WK3 compared to arrival, due to an increase in *Mycoplasmataceae* at WK3 (50-80%) compared to arrival (25-50 %). At WK3 there was an increase in richness and diversity of ARGs compared to arrival in animals that received metaphylaxis, and an increase in diversity of ARGs in animals that received no antimicrobials. Aminoglycoside ARGs were the only class with differences in relative abundance among TxGroups at WK3.
Conclusions
Tulathromycin metaphylaxis increased risk of isolation of MDR *MH*, and in this population, the increase in MDR *MH* appeared to be associated with ICE-containing antimicrobial resistance genes for multiple antimicrobial classes. This study highlights the complexity of assessing the impact of antimicrobial administration on AMR, because, although tulathromycin metaphylaxis increased the risk of isolation of MDR MH and affected the resistome, resistome changes measured did not include differences in macrolide resistance genes, and time and BRD treatment contributed to larger differences in the microbiome and resistome than metaphylaxis.

1117 - Relationship between Thoracic Ultrasonography Findings and Etiology of Bovine Respiratory Diseases in Beef Cattle during the First Month after Arrival

Author: Maud Rouault, Gilles Foucras, François Meurens, Sébastien Assié

Objectives

The study aimed to investigate the relationship between thoracic ultrasonography (TUS) findings and the etiology of bovine respiratory diseases (BRD) in French beef cattle during the first month after they arrive at fattening operations.

Material and methods

This prospective study, conducted from January to June 2023 in nine fattening operations in Western France, received approval from the local Ethics Committee. The animals, predominantly of Charolais (81%) or Limousine (9%) breeds, were clinically assessed (rectal temperature and inspection of BRD signs) weekly, from their arrival until 30 days after arrival. TUS of the right side was performed, focusing on both ventral and dorsal aspects of the 4th and 5th intercostal spaces, resulting in four portions examined per animal. The area (in cm^2) of the most severe lung lesion was recorded for each portion, and the cumulative right lung consolidation area was computed. Nasal swabbing was performed on sick animals (rectal temperature ≥ 39.7°C and at least one sign of BRD) for qPCR identification of *Mannheimia haemolytica (M. haemolytica)*, *Mycoplasmopsis bovis (M. bovis)*, Bovine Coronavirus (BCoV), Bovine Respiratory Syncytial Virus (BRSV), and Bovine Parainfluenza Virus 3 (BPIV-3). Blood samples were also drawn on days 0 and 30 for serological analysis against the aforementioned pathogens. Associations between serological and/or qPCR data and the maximum total right lung consolidation area (MaxRCarea) and/or the maximal number of portions with lung consolidation (MaxRNbC) were analyzed using Fisher's exact test. A logistic regression was used to assess whether these scores could predict positive results for any pathogen. Statistical analyses were performed using RStudio.

Results

126 beef cattle aged 4 to 14 months (average weight = 323 ± 159 kg) were enrolled. Among them, 49 (38.9%) were considered sick at least once, and 32 (25.4%) were treated within the first month after arrival. On the initial examination day, TUS was performed on half the animals, revealing abnormal features in only one. Subsequently, 21 animals showed lung lesions (14 with 1/4 portion injured, 7 with 2/4 portions injured). MaxRCarea was distributed as follows: 0 cm^2 (*n*=105), [1; 4 cm^2] (*n*=7),]4; 8 cm^2] (*n*=6), and >8cm^2 (*n*=8). Concerning etiology, 47.6% of the animals showed a significant increase in antibody titers or positive qPCR results for BCov, 45.2% for BPIV-3, 43.6% for BRSV, 42.9% for *M. bovis* and 42.9% for *M. haemolytica.* Regarding pathogen associations, some animals showed a simultaneous significant increase in antibody titers or positive qPCR results for *M. bovis* and BCoV (24.6%), for *M. bovis* and BRSV (20.6%), and for *M. bovis* and BPIV-3 (17.4%). An association of *M. haemolytica* with BCoV (23.8%), BRSV (22.2%), and BPIV-3 (23.8%) was also detected. Finally, 19.8% of the animals showed evidence of concurrent infection by *M. bovis* and *M. haemolytica*.

Regarding bacteria, significant differences in MaxRCarea and MaxRNbC were observed between animals showing positive results for *M. bovis* compared to those that tested negative. Notably, all the animals having two injured portions or showing lung consolidations > 8 cm^2 tested positive for *M. bovis*. However, according to the logistic regression model, a MaxRCarea exceeding 4cm² could not predict the involvement of *M. bovis* as indicated by qPCR or serological positive results. No significant correlation was established between right TUS findings and the presence of *M. haemolytica*.

Regarding viruses, similar observations were reported for BCoV. Significant differences in MaxRCarea were observed between animals with BRSV or BPIV-3 infection and those that tested negative for these pathogens.
Regarding pathogen associations, there were significant differences in both MaxRCarea and MaxRNbC between animals that tested positive concurrently for *M. bovis*, and either BCoV or BRSV and those that tested negative for any *M. bovis* combination. In the case of the BPIV-3 association, this difference was only significant for MaxRNbC. Interestingly, when TUS results were compared by excluding one of the two pathogens from the association, no significant difference was observed.

Conclusions

Our results demonstrate a positive association between the presence of certain pathogens, notably *M. bovis*, in association with respiratory viruses, and the number and/or the area of lung consolidations as detected by right TUS in beef cattle during the first month of fattening. However, neither the number nor extension of the TUS findings are predictive of the presence of *M. bovis*.

1112 - Assessment of Vaccine Interference Observed Following Co-Administration of Two Modified-Live Viral Intranasal Vaccines to Colostrum-Fed Neonatal Calves in the Face of a BHV1(IBR) Challenge

Author: Michael W. Bolton, Scott Nordstrom, Kevin Hill, Lowell Midla, Philip J. Griebel, Karthic Rajamanikam, Geert Vertenten

Objectives
To compare immune responses of two commercial, modified live vaccines (MLV), IBR, PI3, BRSV (N3) and Bovine Coronavirus vaccine (BCV), when each was given intranasally (IN) alone or co-administered to young colostrum fed Holstein calves. The second objective was to evaluate interference with prevention of infection and/or disease following a BHV-1 (also known as IBR) challenge when the same vaccines were co-administered or given alone.
Material and methods
Thirty-six, male, colostrum fed (200grams IgG from Saskatoon Colostrum Co. within 4 hours of birth), Holstein calves, (4 groups - 9 per group) were gathered from a commercial dairy. All were BVD PI negative and, at about 10 days of age, were vaccinated IN with either vaccine diluent(control), (N3), (BCV), or both vaccines (N3+ BCV) administered in separate nostrils. Calves were housed separately for study duration with no contact between groups. Calves were revaccinated (boostered) 4 weeks later. Nasal secretions and blood were collected prior to enrollment, and weekly during the study. 3 weeks after booster vaccines were given, the calves were challenged with BHV-1 and euthanized 10 days post challenge to quantify total lung pathology.
Results
All calves were seropositive for BHV-1 and BCV prior to vaccination due to presence of colostral antibodies. No significant differences were observed when comparing BCV-specific serum IgG and nasal IgA antibody responses in the BC versus BC+N3 group nor were there any significant differences when comparing BHV-1-specific serum IgG and nasal IgA antibody responses in the N3 versus BC+N3 group. Cytokine responses between groups were also not significantly altered. Nasal BHV-1 shedding after the BHV1 challenge was significantly reduced in both the N3 and BC+N3 groups. Significant reduction in lung pathology was observed when N3+BC group was compared with Placebo and BC groups. Another interesting finding was BC alone significantly reduced lung lesions compared to the diluent (control) group. Also, the BC+N3 group had significantly lower lung lesions than N3 group alone after BHV1 challenge.
Conclusions
This study provides evidence that these two (MLV) IN vaccines (N3 and BCV), can be co-administered to neonatal calves without a significant decrease in the immunogenicity or a reduction in the prevention of BHV-1 respiratory infection and disease. Also of interest, BCV vaccine alone had a significant protective effect against total lung pathology after a BHV1 challenge. This result highlights the role Corona virus may play in total lung pathology, even when the inciting antigen is not Coronavirus. It also gives evidence of a protective effect of Bovilis Coronavirus vaccine against lower respiratory disease in the face of a BHV1(IBR) challenge.

1691 - Addressing BVD endemicity: understanding its prevalence in Mexican herds

Author: Ana Delia Rodríguez Cortez, Lucia E. Rangel, Arantzatzu Lassala, Rogelio Alonso, Carlos G. Gutiérrez

Objectives

To determine the apparent seroprevalence of Bovine Viral Diarrhea (BVD) in Mexico and understand the associated risk factors influencing its spread among cattle populations through a national sampling that considered the cattle population density of each state.

Material and methods

For the seroepidemiological study a sample of 2,686 bovine sera from healthy grazing animals over 2 years old, unvaccinated, and from 430 municipalities and 30 Mexican states was used. The country was divided into 5 regions (North, Gulf, Center, Peninsula, and Pacific) sharing geographic proximity, agroecological conditions, and similar management practices. Samples were diagnosed for seropositivity to the recombinant-E2 protein of the BVD virus using a Luminex® immunoassay previously standardized and validated by our laboratory with 100% sensitivity, 94.7% specificity, AUC= 0.981 (95% CI 0.95-1.0), LR(+) 19.0, and CV R (11.03%) r (4.28%). Logistic regression was used to calculate prevalence, and relative risk (RR) was determined for environmental factors such as temperature, humidity, and climate, as well as production purpose, age of the animals, type of mating, breeding seasonality, and type of grazing.

Results

Regional seropositivities were 50.6% for the North; 57.6% for the Pacific; 46.9% for the Center; 58.3% for the Gulf; and 44.2% for the Peninsula. The levels of seropositivity were considered mesoendemic to hyperendemic with no statistically significant differences between regions of Mexico. Humidity, type of mating, and breeding seasonality affected the prevalences significantly different ($p \leq 0.05$). Subhumid environments showed a prevalence of 59% and RR of 1.15, while very arid environments had a prevalence of 30% and RR of 0.56 ($p=0.031$). Continuous mating had a prevalence of 54% and RR of 1.37, contrasting with seasonal mating's 40% prevalence and RR of 0.73 ($p=0.03$). Artificial insemination (AI) resulted in a 63% prevalence and RR of 1.20, while natural mating had a 5% lower prevalence and RR of 0.95 ($p=0.02$).

Conclusions

The prevalence of BVD in various regions of Mexico ranged from meso- to hyperendemic. These rates, that derived from adult non-vaccinated animals, indicate exposure to pathogens. The high environmental humidity associated with an increased relative risk of seropositivity draws attention to Mexico's coastal areas with higher antibody titers. Similarly, continuous breeding and artificial insemination are associated with increased seropositivity risks compared to their counterparts. The high seropositivity in grazing cattle herds in Mexico is concerning, especially as the global trend moves toward the control or eradication of BVD to prevent disease reemergence, contain its spread, mitigate economic impact, and prevent trade limitations. Therefore, it is vital to address BVD endemicity with effective vaccines, establish antibody diagnostic techniques for segregating highly positive animals, and develop diagnostic techniques for detecting persistently infected animals, who make up to 3% of herds and serve as the primary viral reservoir within production systems.

1795 - Loop-Mediated Isothermal Amplification (LAMP) for the diagnosis of bovine diarrhea virus

Author: Fernando Cerón Téllez, Elizabeth Loza-Rubio, Marisela Leal-Hernandez, Sabrina Polo-Contreras

Objectives

The beef and dairy cattle industry constitutes the main means of production of animal protein in Mexico. The sources of economic losses in cattle are diverse and are related to diseases caused by viruses. Within these diseases, one of the most important is bovine viral diarrhea (BVD) since it is an endemic disease with mandatory notification in Mexico. Due to the importance and impact of the disease, there is a need for diagnostic methods for rapid identification. and elimination of carriers in herds. New diagnostic tools such as Loop-Mediated Isothermal Amplification (LAMP) have been developed. The objective of this work was to develop the LAMP test to detect the bovine viral diarrhea virus in cattle samples.

Material and methods

The bioinformatics design of three pairs of primers was carried out with the NEB® LAMP primer design tool, using as a reference the nucleotide sequence of the gene that encodes the E2 protein of the DVB virus with the GenBank accession number JX419398.

To obtain a positive control, RNA extraction from the Bovi-Shield GOLD® FP®5 VL5 vaccine was done using the Invitrogen-Viral RNA/DNA® Kit, which was then subjected to RT-PCR using the kit. One Step Qiagen. Amplification at 214 bp was verified by electrophoresis in a 1.5% agarose gel. Positive control for LAMP was obtained doing different concentrations; these concentrations were subjected to LAMP to observe their amplification times. The colorimetric results were confirmed by electrophoresis in a 3% agarose gel.

10 vaginal and 10 nasal swabs were chosen, from Aguascalientes state, collected in 2020. RNA extraction from samples was done with FAVORGEN® FavorPrep™ Viral Nucleic Acid Extraction Kit I (with carrier RNA-low viral load) was used.) - FAVNK-001-2.

RT-PCR from different samples was carried out with Qiagen One Step kit and the conditions established for DVB amplification were used. For the LAMP reaction, the WarmStart® Colorimetric LAMP MasterMix with UDG was used, in which 5 µL of RNA from each sample was used and 3 µL was used for the positive control.

For the LAMP technique, the positive control presented a correct colorimetric amplification and this was obtained at 60 minutes; Therefore, this concentration and average time were established as standard. The results could be observed with the naked eye and were confirmed by electrophoresis in a 1.5% agarose gel at 90 volts.

Results

We had 19 positive samples and one negative using this colorimetric test; these result concorded with results obtained by RT-PCR. Based on this, calculations were made, giving a sensitivity of 100% and a specificity of 94%, although it is necessary to use a greater number of samples so that this sensitivity and specificity are verified without a doubt.

Some authors such as Mungthong, K. et al (2021) have reported similar sensitivity and specificity results (100% and 100%, respectively) with RNA samples for the same virus. The specificity of 94% was due to having a negative sample in both techniques, however, it is still a high percentage, which if the validation were carried out with more samples could be analyzed in a better way.

Conclusions

LAMP demonstrated better results in terms of time and detection limit than RT-PCR. Its sensitivity and specificity demonstrate the efficiency of detecting true positives and negatives, indicating that it is a reliable test to be used in the field. This can be a viable option since it is a technique that can be performed without problems anywhere without having to send the samples to a laboratory; However, it is necessary to test more samples in order to validate these results in comparison with reference techniques.

1150 - Prevalence, biosecurity and risk management of bovine coronavirus infections on white and rose veal and dairy source beef calf farms in Europe.

Author: Anna Catharina Berge , Geert Vertenten

Objectives
The objective of this study is to obtain a rough estimate of the farm prevalence of Bovine Corona Virus (BCoV) in dairy source slaughter calf production in European countries and to characterize farm-level risk factors in management and biosecurity that are linked to calf coronavirus infection.
Material and methods
This is a cross-sectional field study of over 70 farms raising dairy-source calves for meat in Europe, based upon a convenience selection of farms. The farm enrolment was in proportion to the national production. Farms were visited one to two times for sample collection and herd information. On every farm, 10-20 calves recently arrived at the farm, 10-20 samples recently weaned calves or white veal calves more than 6 weeks on the farm and one faecal slurry sample were collected. Nasal and faecal swabs and slurry samples were collected for BCoV detection using semi-quantitative Real Time PCR (RT-PCR), and blood serum samples were collected for BCoV antibody detection using ELISA. Antibodies to other respiratory pathogens were also evaluated. A questionnaire was performed to determine husbandry and management factors associated with BCoV prevalence. Biosecurity was scored using the Biocheck (https://biocheckgent.com/). Data is entered in excel and analysed with SAS version 9.4. Multiple logistic regression models (binary and ordinal) with random effects of herds is used to evaluate relationship between husbandry, management, and biosecurity on farms.
Results
The study is ongoing, and we have preliminary analysed results from 49 farms in Belgium, Denmark, France, Hungary, Italy, Netherlands, Romania, Spain, Sweden, and the United Kingdom. BCoV viral material was isolated in 100% of farms. The median farm had 71% BCoV-positive calves. Presence of BCoV viral material was confirmed in 70% of nasal swabs and 67% of faecal swabs. Thirty percent of the calves had no positive samples, 23% of calves had either a positive faecal or nasal swab sample, and 47% of calves had both faecal and nasal samples being BCoV positive. In the sampled farms, the recently arrived calves exhibited the highest levels of positivity on PCR (low CT-values) with 91% of calves being BCoV PCR positive, and 47% of the weaned/older calves being BCoV PCR positive. Seventy-nine percent of slurry samples were BCoV PCR positive. Median antibody levels (measured by optical density) were 40-60 (++) in preweaned calves and 60-80 (+++) in weaned/older calves. The biosecurity scores are overall low on the farms in this study (pending analysis at the time of abstract submission). Risk factor analysis will be presented.
Conclusions
The preliminary results indicate that BCoV is intensively spreading in dairy source calves intended for slaughter. After arrival at the farm most calves are spreading virus in both the respiratory and enteric pathway and high levels of virus transmission are also seen in weaned or older calves.

Parasitic diseases

1285 - Mezquite pod as a potential nutraceutical for the control of >>Haemonchus contortus<<

Author: Sara Atzín Muñoz Marín, Cintli Martínez-Ortiz-de-Motellano , Claudia Cecilia Márquez Mota, Laura González Reyes, Augusto César Lizarazo Chaparro, Gloria Sarahí Castañeda Ramírez

Objectives

The aim of the study was to demonstrate the anthelmintic effect of an ethanolic extract derived from *Prosopis laevigata* pods.

Material and methods

The Larval Migration Inhibition Assay (LMIA) was performed in the laboratory of the Department of Animal Nutrition and Biochemistry. From a plant extract of *P. laevigata* (mesquite) pod, solutions were prepared at concentrations of 25, 50, 150, 250, and 350 µg/ml with PBS. Levamisole solutions (Levamisole 5% SIGMA) were prepared with PBS at concentrations of 1%, 0.5%, 0.25%, and 0.125%. Ivermectin solutions (SIGMA) were prepared with DMSO at concentrations of 1%, 05%, 0.25%, and 0.125%. All solutions were stored at 4 °C, and two hours before use, they were brought to room temperature. Incubation was conducted in 50-mL Falcon tubes. In each tube, 700 *Haemonchus contortus* larvae were concurrently incubated with varying concentrations. They were incubated for 3 hours at room temperature. After incubation, the falcon tubes were incubated to remove the compounds from the plant extracts, levamisole and ivermectin. The L3 were centrifuged at 2500 rpm for 3 minutes and washed with distilled water; this step was repeated twice. In 48-well cell culture plates, 0.5 ml of PBS was deposited in each well. Subsequently, the inserts were placed with a 25-micron nylon mesh, checking the permeability between the mesh and the PBS. Then, 0.5 ml of larval solution was placed, which had already been exposed to different concentrations of the plant extract or to different concentrations of levamisole or ivermectin. After 3 hours of incubation at room temperature, the inserts with the nylon mesh were carefully removed to recover the solution from each well. These solutions were poured into previously identified 2 ml tubes with the concentrations of plant extract, levamisole, ivermectin, and PBS for subsequent reading. A one-factor analysis of variance (ANOVA) was used to identify the variance between the different treatments. The response variables were the different concentrations of each treatment.

Results

The ethanolic extract of mesquite pods demonstrated effective inhibition of larval migration. The highest inhibition, reaching 99%, was observed at a concentration of 150 µg/mL. Subsequently, concentrations of 250 µg/mL and 350 µg/mL demonstrated inhibitions of 96.5% and 94.49%, respectively. In comparison, positive controls revealed that anthelmintic treatment with levamisole consistently achieved an inhibition percentage exceeding 98%. Additionally, the ivermectin treatment displayed a notable inhibitory effect, with a percentage of 95% at the 0.5% concentration and even higher percentages at other concentrations (1%).

Conclusions

The impact of nematodosis is one of the most frequent causes of economic losses in production systems. Substantiated by the decrease in daily weight gain (up to 50%) and mortality rates (20–50%), in addition to the costs associated with veterinary interventions and treatments. *H. contortus* is a hematophagous nematode capable of clinically and sub-clinically affecting the young and adult sheep population. Cases of anemia are common due to severe blood loss, while low feed conversion is observed in production animals, and there is often high mortality in young animals. The control of gastrointestinal nematodes is based on the use of broad-spectrum drugs of chemical origin. The indiscriminate and irrational use of available chemical treatments has originated the phenomenon of anthelmintic resistance, which has been reported worldwide and affects small ruminant producers. *Prosopis laevigata* represents a natural resource of great importance for the inhabitants of the arid and semi-arid zones of Mexico, since it serves as fodder for domestic and wild animals due to its nutritional content.

The results obtained in this study successfully fulfilled the proposed objectives. This is proof of the *in vitro* anthelmintic effect of an ethanolic extract derived from mesquite pods. Also, this is particularly significant given that prior studies predominantly concentrated on employing the leaves and/or stems of the same tree.

The LMIA demonstrated the significant efficacy of the ethanolic extract from mesquite pods. Particularly at concentrations of 150 µg/mL and above, in comparison to the negative control. Any misuse of the results presented here remains the responsibility of the reader and not of the authors. Further studies are needed to be able to continue with the necessary recommendations for the use of this plant as a nutraceutical.

1264 - Comparison of single versus combination dewormers on fecal egg counts and weight gain in young grazing cattle

Author: Gabriele Maier , Josh Davy, Larry Forero, Jeffery Stackhouse, Grace Woodmansee

Objectives
The objective of this study was to quantify the effect of a combination treatment with two classes of anthelmintics (macrocyclic lactones and benzimidazole) versus treatment with only one class of dewormer (macrocyclic lactones) on fecal egg counts and weight gain in growing beef cattle on pasture.
Material and methods
Grazing cattle between 6 and 18 months of age (N = 286) from six beef herds in northern California with no history of deworming in the past 45 days or not in the previous 150 days with long-acting eprinomectin were randomly assigned to receive either a single subcutaneous injection of 200 mcg ivermectin [Ivomec Plus, Boehringer Ingelheim Animal Health, Duluth, GA] per kg body weight or a combination of the same dose of subcutaneous Ivomec Plus and one oral dose of fenbendazole at 5 mg per kg body weight [Safeguard, Merck Animal Health, Rahway, NJ]. Fecal samples of approximately 50 g were collected per rectum on day of enrollment and approximately 14 days later (13 – 20 days range) from 20 cattle in each group and assessed for the presence of trichostrongyle type eggs with the Mini-FLOTAC system. Fecal egg count reduction was calculated on the top ten shedders in each group using the online calculator shiny-eggCounts with Bayesian hierarchical modeling. Coprocultures were also performed on a pooled sample from each treatment group within a herd at both time points. Body weights were measured on day of enrollment and after 2 – 4 months of grazing with both groups commingled and average daily gain analyzed in a general linear mixed model with herd as the random effect and adjusted for cattle age and pasture type (irrigated pasture or dry range).
Results
Two herds had fecal egg counts below the minimum threshold of 200 eggs across 10 animals per treatment group recommended by the European Cooperation in Science and Technology and were eliminated from the analysis. Ivermectin treatment alone led to fecal egg count reductions between 28 % (UI 12 – 58 %) and 68 % (UI 43 – 82%) in four herds indicating resistance to this class of dewormer. On the other hand, the combination of ivermectin and fenbendazole led to fecal egg count reductions between 97% (UI 91 – 100 %) and 100 % (UI 96 – 100 %) in commingled herdmates. The most commonly observed genus of nematode L3 larvae in coprocultures was *Cooperia* in three of four herds before treatment and in all four herds post treatment where larvae could be recovered. There was no statistically significant difference in the average daily gain of cattle in the control group (0.62 kg ± 0.21) and the treatment group (0.65 kg ± 0.29, P = 0.56).
Conclusions
There may be widespread resistance to the macrocyclic lactone class of anthelmintics in grazing beef cattle in northern California while treatment with a combination of macrocyclic lactones and benzimidazole still achieves appropriate internal parasite control. Helminths in the genus *Cooperia* appear to be driving resistance. Despite marked differences in fecal egg counts post treatment, there was no difference in weight gain detectable between the two study groups. Testing of parasite loads prior to treatment could improve the judicious use of dewormers in this population.

1181 - Growth performance of beef heifers treated with an injectable fixed-dose combination (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) or single-active (0.2 mg/kg ivermectin) endectocide

Author: Andrew DeRosa, Jase Ball , Susan Holzmer, Landon Watkins, Mitchell Blanding, Mark Alley, Thomas Short, David Bechtol, Audie Waite, Elizabeth Rigoni, Jezaniah Tena, Frederico Moreira

Objectives
Gastrointestinal nematodes (GINs) can negatively impact all production classes of cattle, particularly growing cattle. A global decline in efficacy of broad-spectrum single-active anthelmintics requires alternative GIN control methods without the aid of novel drug classes.
Material and methods
Here, we present a new fixed-dose combination injectable (FDCI) endectocide for cattle that combines doramectin (5 mg/ml) and levamisole hydrochloride (150 mg/ml). A 56-day comparative performance confinement backgrounding trial was conducted in stocker beef heifers (n = 1548) with confirmed GIN infections to (1) compare the Day 14 post-treatment effectiveness of the new FDCI endectocide to pen mates treated with the injectable single-active endectocide ivermectin, as evidenced by fecal egg counts (FECs) conducted for a randomly selected subset (10%) of both treatment groups, and (2) determine if the greater GIN control by the FDCI evidenced in the subsample improved growth performance in all FDCI-treated heifers. Heifers were procured in four cohorts, with a 10-week timeframe between enrollment of the first and last cohort. Treatment groups were comingled within dirt-floor pens (n = 31; 7 to 8 per cohort) and offered a standard backgrounding diet *ad libitum* for the study duration. Heifers with enrollment FEC ≥ 30 eggs per gram (EPG) were randomly allocated to receive the FDCI (n = 773) or ivermectin (n = 775) on Day 0.
Results
Day 0 FECs conducted on 10% of enrolled heifers (FDCI, n = 78; ivermectin, n = 79) were not different between treatment groups (p = 0.491). Day 14 FECs for the same heifers were reduced compared to Day 0 within each treatment group. Heifers given the FDCI had lower Day 14 AM FECs and higher FEC reduction test (FECRT) result (0.07 EPG; FECRT = 0.999) than ivermectin-treated heifers (21.58 EPG; FECRT = 0.850). Mean body weight (BW) was not different between treatment groups on Day 0 (p = 0.2762) and Day 14 (p = 0.2010) but was significantly greater (p = 0.0007) for FDCI-treated heifers compared to ivermectin-treated heifers on Day 56. Compared to ivermectin-treated heifers, overall average daily gain from all evaluation periods (Day 0 to 14, Day 14 to 56, and Day 0 to 56) was greater (p ≤ 0.0052) in FDCI-treated heifers, and FDCI-treated heifers had 4.223 kg greater total weight gain over the 56-day study.
Conclusions
The FDCI (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) was highly effective in reducing GIN infections and thus promoted improved growth performance in beef heifers over a 56-day backgrounding period.

1077 - Injectable trace minerals associated with vaccination against Haemonchus contortus program reduce worm egg counts and increase average daily gain in lambs in Australia, a pilot study

Author: Paula Gonzalez-Rivas , George Cox , Timothy Elliott

Objectives

Gastrointestinal nematodes cost the Australian sheep industry $369 million annually. Barber's pole worm (*Haemonchus contortus*), a blood-feeding nematode that parasitises the abomasum, is one of Australia's most important sheep roundworms. Immunity to *H. contortus* is achieved not earlier than 18-24 months old in sheep, and lambs under six months of age cannot mount an effective immune response due to a delay in developing acquired immunity to the parasite (Colditz et al. 1996).

Vaccination against barber's pole (Barbervax®) and oral trace mineral supplementation have been evaluated to improve the host's resistance to gastrointestinal parasites. Injectable trace minerals (ITM) containing Cu, Se, Zn and Mn allow the delivery of specific amounts of trace minerals, by-pass antagonist interactions within the gastrointestinal tract, and overcome variability associated with fluctuations in voluntary intake of free-choice mineral mixes. ITM complement oral supplementation and support challenging events of the productive cycle (weaning, joining, pregnancy, transport) by enhancing livestock antioxidant system, immunity, and fertility. Studies have demonstrated that ITM can improve vaccination responses against viral diseases in cattle (Arthington & Havenga 2012; Palomares et al. 2016; Bittar et al. 2020), suggesting that adding ITM to livestock management protocols might represent a promising tool to improve health on commercial farms. However, the effects of ITM associated with a vaccine against parasites have not been investigated. This study hypothesised that ITM associated with Barbervax® vaccination protocol in lambs could reduce *H. contortus* worm egg counts (WEC) beyond what is expected with the vaccine alone.

Material and methods

Sixty lambs were allocated to two treatment groups based on body weight at marking. All lambs received a Barbervax® vaccination program following recommended protocols (wormboss.com.au). From weaning, 30 out of 60 lambs received an ITM product containing Zn, Mn and Se (Multimin® Copper-free for Sheep and Cattle, Virbac Australia Pty Ltd) every three months until the last vaccine dose (V6). All lambs received parasiticides at weaning to control scour worms and early barber's pole worms and received clostridial vaccination at marking and a booster at weaning. Lambs were weighed every 14 days from marking, and faecal samples were collected every 14 days from weaning for individual WEC and larval differentiation. Bodyweight (BW) data were analysed using ANOVA in GenStat, and *H. contortus* WEC data was analysed using the Chi-square test in MedCalc. Significance was defined as $P < 0.05$. The efficacy of the treatment was calculated as Mean EPG (eggs per gram) of control – Mean EPG of treatment / Mean EPG of control x 100.

Results

This pilot study demonstrated that the use of ITM every three months from weaning associated with the Barbervax® vaccination program resulted in a significant reduction of *H. contortus* and higher efficacy (between 41 to 85%) at days 73, 110, and 136; $P < 0.05$ compared to control animals. In addition, lambs treated with ITM had higher BW (+1.72kg) and ADG at 181 days (128 vs 141g/day $P < 0.05$).

Conclusions

It is suggested that ITM can increase the immune response to barber's pole vaccination. Further studies can help to understand the mechanism behind those results, i.e., antibody responses, change in antioxidant status and trace mineral levels, and specific markers for *H. contortus.*

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1288 - Targeted selective treatment at housing with injectable eprinomectin in French dairy cattle using a validated decision support system (TRI)

Author: Vincent Jégou, Bertrand Maynard, Céline Cotrel, Damien Achard

Objectives

Anthelmintic resistance (AR) is a reality across Europe that threatens the sustainability of the cattle industry. Until recently, cattle farms were not considered at risk for AR but several reports have confirmed its presence in many countries. To limit the development of AR, it is necessary to rethink our views on strategic "systematic" program. Targeted selective treatments (TST) are potentially good options but their adoption in cattle herds has been limited due to low level of awareness, fear of economic losses, and difficulty to implement them. To encourage acceptance of TST in French dairy cattle herds, a decision support system (TRI) based on validated criteria was developed that gives farmers access through their veterinarian to a list of dairy cattle to be treated at housing with eprinomectin injectable (Eprecis® 20 mg/mL).

Material and methods

In participating dairy herd and with farmer's consent, the following data is collected for each lactating cows: ID, individual milk production, days in milk (DIM) and parity. Key findings from a previous study in 120 French dairy herds were used to generate the TRI list (Ravinet *et al.*, 2018). Specifically, best responding cows were found to had calved during the grazing season (less than 200 DIM at the time of treatment) and to have a low-to-medium peak performance for their class of parity. Based on the farm data and these two individual criteria, a list is generated indicating the dairy cows eligible for treatment and the economic impact of different interventions (from systematic deworming to selective deworming).

Results

Since its launch in 2021, the TRI initiative has been adopted by more than 1 000 dairy herds and contributed to decrease the use of eprinomectin by 75% while increasing farmer's revenue by 30%.

Conclusions

Refinement of the use of anthelmintics is possible using validated scheme and active collaboration across the industry. In the future, this dedicated decision support system (TRI) will be automated and aim to indicate the level of environmental exposure to endectocides.

1111 - The effect of Potassium Sorbate on Crypto GP40 antibodies in colostrum

Author: Geert Vertenten ., H. Swam, Nigel Gulickx, Jules Petit, Bart Sustronck, Mark van Roosmalen

Objectives

A recently launched vaccine (Bovilis Cryptium, MSD Animal Health) stimulates active immunity of pregnant heifers and cows by raising antibodies in their colostrum against Gp40 of *Cryptosporidium parvum,* intended for passive immunisation of calves to reduce clinical signs (i.e. diarrhoea) caused by *C. parvum*. Potassium sorbate (PS), a widely used food preservative, has shown antimicrobial properties against various microorganisms. However, its effect on Gp40 antibodies in bovine colostrum following vaccination with Gp40 antigen vaccine has not been examined. This study aims to investigate the impact of potassium sorbate on the stability and functionality of *Cryptosporidium* Gp40 antibodies in bovine colostrum obtained from cows vaccinated with the Gp40 antigen vaccine.

Material and methods

A 50% solution of PS in water for injection (WFI) was prepared : 1g PS (CH-886, Merck KGaA Life Science, the Netherlands) was supplemented to 2ml WFI. Frozen colostrum samples from an efficacy trial with the cryptosporidiosis vaccine (Timmermans et al. 2023) were obtained. Those samples were thawed at 4°C the day before use. Four samples were used from 3 categories (low-middle-high Gp40 colostral antibody titer). Each sample was tested under 4 conditions: room temperature without mixing of PS, room temperature with PS, 4°C without PS and 4°C with PS. When PS was added, 20µl of the 50% PS solution was added to 1.98ml colostrum to obtain a 0.5% PS end concentration. The colostrum samples were tested with an *in house* Gp40 Elisa (Center for Diagnostic Services, Boxmeer, The Netherlands) on day0 (d0), d1, d2, d3, d4, d8 and d10. The statistical analyses were performed using a three-way repeated measures Anova, conducted in R (R core team 2022) with $p<0.05$.

Results

Three-way interaction, two-way interactions and main effects all are non-significant. So, there is no significant influence of time ($p=0.112$), storage condition ($p=0.769$) or addition of potassium sorbate ($p=0.127$) on the Gp40 concentration in bovine colostrum. An important finding was that 5 of the 18 samples that were stored at room temperature became solid, indicating spoilage due to bacterial growth. Four of the five spoiled samples did not contain PS, further supporting the anti-microbial properties of PS in bovine colostrum.

Conclusions

The addition of a potassium sorbate solution to colostrum has no negative effect on the amount of *Cryptosporidium* Gp40 antibodies in colostrum. In this trial, it has been reconfirmed that the addition of potassium sorbate is an effective way to conserve colostrum at room temperature.

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1495 - Pharmacodynamic Interactions in Artemisia cina Ethyl Acetate Extract: Anthelmintic Effects on Haemonchus contortus Eggs and Infective Larvae L3

Author: Luis David Arango-De la Pava, Rosa Isabel Higuera-Piedrahita, Jorge Alfredo Cuéllar-Ordaz, Manasés Gonzaléz-Cortazar, Raquel López-Arellano, Alejandro Zamilpa

Objectives
Establish the presence of pharmacodynamic interactions between the major chemical compounds of the ethyl acetate extract of *Artemisia cina* with anthelmintic effect on *Haemonchus contortus* eggs and infective larvae L_3 using binary mixtures.
Material and methods
Fresh pre-flowering leaves and stems of *A. cina* were acquired from [Hunab], a greenhouse cultivator of the plant. The plant material was air-dried in the absence of light, ground, and subjected to extraction using ethyl acetate through maceration at room temperature for 48 hours. The resulting liquid extract was filtered (No. 4 Whatman filter paper), concentrated to dryness at 40 °C under low pressure, and finally lyophilized. This extract was stored at 4 °C for subsequent phytochemical and biological assays. The extract underwent separation through open column chromatography, utilizing normal silica gel 60 (Merck, 0.015-0.040 mm) as the stationary phase and an ethyl acetate-n-hexane solvent gradient system, monitored with thin-layer chromatography (TLC). Isolated compounds were then assessed for anthelmintic activity in larval mortality (LM) and egg hatching inhibition (EHI) assays, conducted in vitro in a 96-well microplate with approximately 100 L_3 larvae/eggs per well. Compounds displaying anthelmintic activity were further identified through HPLC-DAD, UPLC-MS, and one- and two-dimensional NMR experiments (^{1}H, DEPTq COSY, HSQC, HMBC). Based on the observed LM and EHI anthelmintic activity, a lead compound was selected for binary mixture studies. These binary mixtures were evaluated using a fixed proportion scheme, and differences among lethality percentages were compared using the Tukey test ($p < 0.05$). Lethal concentrations (LC_{25}, LC_{50}, and LC_{90}) were determined utilizing the PROBIT procedure.
Results
According to TLC, three compounds (C1, C2 and C3) were isolated from the ethyl acetate extract in sufficient quantities to perform biological assays and structural elucidation. C2 and C3 were analyzed using HPLC, obtaining peaks at different retention times and UV spectra between λ 205 and 217 nm, confirming that were isolated compounds. C1 was analyzed by CG-MS, resulting in a mainly 2-compounds a mixture. According to 1D-2D RMN analysis, C1 is a mixture of 52.51% hentriacontane and 21.34% of 1-nonacosanol, C2 cinic acid and C3 peruvin. C1 presented the best EHI (LC_{50} 0.45 (0.42-0.49) mg/mL and LC_{90} 1.32 (1.21 - 1.45) mg/mL), followed by C3 (LC_{50} 1.09 (1.05-1.13) mg/mL) and LC_{90} 1.58 (1.50 - 1.68) mg/mL). C2 did not present LC_{50} o LC_{90} in the range of concentrations evaluated (2-0.01 mg/mL). In LM C2 presented the highest larvicidal activity (LC_{50} 0.01 (0.01 - 0.03) mg/mL and LC_{90} 0.012 (0.19 - 0.25) mg/mL, followed by C3 (LC_{50} 0.12 (0.13-0.14) mg/mL) and LC_{90} 0.42 (0.37 – 0.40) mg/mL) and finally C1 (LC_{50} 0.06 (0.03 - 0.08) mg/mL and LC_{90} 2.60 (1.81 - 3.43) mg/mL)). Due to the anthelmintic activity on L3 and *H. contortus* eggs, C3 was chosen as leader compound to perform the binary mixtures. The binary mixture C3 $0.5LC_{25}$-C1 $0.5LC_{25}$ and C3 $0.5LC_{25}$-C2 $0.5LC_{25}$ presented synergism, 4.87-fold and 1.25-fold in LM. In EHI, the binary mixture C3-C1 presented additivity in all proportions.
Conclusions
The binary mixtures of peruvin–hentriacontane/1-nonacosanol and peruvin–cinic acid exhibited synergism in the larval mortality of *Haemonchus contortus* infective larvae L_3. Additionally, 1-nonacosanol, hentriacontane, and peruvin are reported for the first time in *Artemisia cina*.

Acknowledgments

This research was funded by the Support Program for Research and Technological Innovation Projects (PAPIIT-UNAM), titled: Evaluación del efecto tóxico del extracto *n*-hexánico de *Artemisia cina* y cinaguaiacina sobre los parámetros bioquímicos en sangre y alteraciones anatomopatológicas en ratas Wistar después de su administración por vía oral (IA204822). Arango-De la Pava was supported by Postdoctoral Grants Program from National Autonomous University of Mexico.

1287 - Efficacy of an oral solution of paromomycin for the treatment of suckling beef calves with cryptosporidiosis in a multicentric field study

Author: Damien Achard , Gaelle Pagny, Charlotte Billy, Jean-François Collin, Anne Trotel

Objectives

Cryptosporidiosis is a frequent parasitological infection of mammals including but not limiting to humans and neonatal ruminants. In newborn calves, cryptosporidiosis has been repeatedly identified as a major contributor of neonatal diarrhoea, a dominant calfhood disease with detrimental health and economic consequences.

Current treatment options for clinically affected newborn calves are limited. A recent meta-analysis work reported halofuginone and paromomycin as valuable oral treatment options while recognizing that halofuginone can present important safety issues and that data is insufficient to fully support the use of paromomycin (*Brainard et al.,* 2020, 2021). To address this lack of evidence, a study under field conditions was performed in beef calves clinically affected by cryptosporidiosis comparing a new dose regimen of an oral paromomycin solution (Gabbrovet Multi®, Ceva Santé Animale) to a reference product based on halofuginone (Halocur®, MSD).

Material and methods

A GCP compliant study was performed according to a multicentric, blinded, positively controlled and randomised design in 40 commercial suckling beef farms located in France and Belgium. The farms were enrolled based on the presence of cryptosporidiosis through positive laboratory or strip testing, absence of recent or current BVD infections, and no recent history of diarrhoea due to coronavirus, *E. coli* and rotavirus, or a dedicated vaccination.

Each newborn calves in this study were required to meet the following criteria for enrollment: age between 3-14 days on first treatment, faecal score ≥ 2, negative rapid test for *E. coli*, coronavirus, rotavirus, and positive rapid test for *C. parvum*. Any calf that presented with diarrhoea for > 24 hours or that has been previously exposed to antibiotics, parasiticides or probiotics was excluded. Animals were randomly allocated to two treatment groups. Calves in group A received 150 mg paromomycin sulfate/kg b.w. (Gabbrovet Multi®), once daily, for 5 days by oral route while calves in group B were orally administered 100 µg halofuginone/kg b.w. (Halocur®), once daily, for 7 days. The following clinical and parasitological parameters were monitored at fixed times during the 21 days of the study: faecal score (0-3), general health observation (0-3), hydration score (0-3), and oocyst counts (number of oocysts per gram of dry faeces). Percentages of calves cured at day 8 was the main criteria to evaluate the efficacy in both treatment groups. Other criteria such as time to clinical cure, number of days when calves were asymptomatic from Day 0 to Day 8 and the parasitological cure at day 7 (oocyst count = 0) were also assessed. The statistical unit was the calf.

Results

One hundred nighty-one suckling calves of beef or crossbred origin from 40 different commercial farms, with a median age of 7 days and an initial mean body weight of 47.7 kg were enrolled. 95 calves were allocated to treatment group A, 96 in the treatment group B. At inclusion, the treatment groups were found clinically comparable.

91.5% (86/94) of newborn calves in group A and 68.1% (64/94) in group B were considered clinically cured at day 8. Clinical cure rate in group A was found superior to those observed in group B ($p<0.001$). In addition, the time to first cure was significantly shorter in group A (log rank test, $p=0.0028$) than in group B. Considering the clinical score on D0, probability of being cured at any time was 48% higher in group A than in group B. The parasitological cure on Day 7 was 16% higher in group A *vs.* group B, with a rate of 79.3% (73/92) and 63.7% (58/91) respectively ($p=0.0466$). In group A, a median of 7 days with normal clinical scores was recorded, whereas in group B, a median of 5 days was reported. In each group, serious adverse events have been

reported for 6 calves. Adverse events were considered as possibly/probably related to the product for 2 calves in group B and none in group A.

Conclusions

In this multicentric field study, daily oral treatment with 150 mg/kg of paromomycin (Gabbrovet Multi®) for 5 days was found safe and highly effective to cure sick beef suckling calves with cryptosporidiosis and to control their oocyst burden. In addition, this new treatment regimen was found superior to the current reference treatment based on halofuginone.

Pharmacology and toxicology

1199 - Evaluation of the healing process of lung lesions following treatment with florfenicol and meloxicam in veal calves affected by bovine respiratory disease during one production cycle

Author: Anastasia Lisuzzo, Damien Achard, Alessio Valenza, Luca Cozza, Eliana Schiavon, Giacomo Catarin, Fabio Conte, Barbara Contiero, Enrico Fiore

Objectives

Bovine respiratory disease (BRD) is a major disease affecting the veal calf industry. Its diagnosis relies on clinical scores reflecting clinical examination (CE) of respiratory signs. However, CE low sensitivity and specificity can delay diagnosis and caused treatment failure. Furthermore, CE is not indicative of the healing process in consolidated lungs following treatment. In contrast, lung ultrasonography (LUS) has gained recognition as performant tool to detect BRD that can be used to document the healing process.

To better characterize the occurrence of BRD during veal calves' production cycle and document the effects of treatment on lung healing process, a field study was performed using LUS as investigation tool and a combination of florfenicol and meloxicam for treatment.

Material and methods

Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. A single stock of 84 veal calves were enrolled with an average age of 30.6±9.6d at arrival. CE and LUS examinations were performed twice weekly for the first 60d and then weekly until the end of the production cycle (180d). Nasal and ocular discharges, rectal temperature, cough, ear position, and abnormal breathing were assessed to calculated two clinical scores: Wisconsin (BRD≥4) and California (BRD≥5) Scores. LUS evaluations were performed in six lung's regions (cranial 4^{th}–3^{rd} intercostal space (ICS), middle 6^{th}–5^{th} ICS, and caudal 10^{th}–7^{th} ICS regions; right and left side). The LUSs were used to establish ultrasonography score (US; 0-5 points score) and modified lung lesion score (LLS; BRD≥10.5). Lung consolidations were measured to provide thickness (cm) and area (cm^2) of each lesion. The sum of all consolidated areas provided the total lung consolidation area (cm^2).

Animals with the US≥3 or consolidation thickness ≥3cm on cranial region were treated with one-shot of florfenicol and meloxicam (40mg/Kg+0.5mg/Kg; Zeleris®, Ceva Santé Animale). Treated group was monitored at +1, +3, +5, +7, +9, +11, and +14d post-treatment. The non-treated animals during the production cycle were classified as control group (CTR).

Differences over production cycle, clinical and LUS follow-ups were assessed by PROC GLIMMIX procedure of S.A.S.-software using the effect of time, groups, sex, breeds, and animal (random and repeated effect). Groups comparisons were performed at arrival, treatment days (for CTR group were used the examination at the same date of treated animals), and at the day before slaughter of each animal. A post-hoc pairwise comparison was performed using Bonferroni correction. A *p-value*<0.05 was accepted.

Results

Thirty-six and 48 calves were included in TRT and CTR groups, respectively. At their arrival, clinical scores and LUS were comparable between groups. Clinical scores, US, LLS, and total lung consolidations were significantly higher in TRT *vs*. CTR calves at the treatment day. At the end of the study, clinical scores and LUS examinations were similar or marginally different between groups. In addition, growth performances and beef quality were similar in both groups (p≥0.23). A vast majority of BRD cases (88.9%) occurred within first 30d after arrival with another 11.1% occurring until 60d. Overall BRD treatment success rate was 94.3%. BRD chronicity rate was 2.9%; and fatality rate was 2.9%. Interestingly in TRT calves, overall clinical scores were not

indicative of disease at treatment day (Wisconsin 2.1±0.4; California 2.8±0.5), but a peak was observed at +5d after treatment (Wisconsin 4.5±0.6; California 5.7±0.8). US, LLS and total lung consolidation were high at the day of BRD diagnosis (US=4.7±0.3; LLS=15.6±1.9; total lung consolidation 30.06±1.98cm^2) with cranial regions showing the largest lesions as determined by consolidation thicknesses and measurements of the different consolidated areas. Following treatment, a swift lung healing process was observed with significative decrease in US at +3d (US=3.64), +5d (US=2.41), +11d (US=1.68), in LLS at +1d (LLS=12.52), +5d (LLS=6.98), +7d (LLS=4.33) and in the total lung consolidation at +1d (15.56cm^2), at +5d (8.57cm^2), and at+9d (3.26cm^2).

Conclusions

In this field study in veal calves, BRD mainly occurred within the first month after arrival. Systematic LUS examinations (US and LLS scores) at multiple timepoints during the production cycle allowed to detect BRD 5 days before the clinical scores which ensured prompt treatment. The evaluation of the lung healing process also revealed the fast and beneficial effects of florfenicol and meloxicam (Zeleris®) in affected calves.

1003 - A pharmacological perspective on the use of Benzyl-penicillin G in production veterinary medicine in Latin America

Author: Itzcoatl Felipe Aquino Díaz

Objectives
A compilation of data from specialized databases on the generalities, spectrum, therapeutic recommendations, safety, PK/PD relationship, interactions, and the legal status of benzylpenicillin-G (BP-G) and its different salts in Latin America is presented.
Material and methods
The data search, classification, and analysis were mainly made from scientific journals and textbooks derived from databases such as Elsevier, PubMed, MedLine, Agricola, and Scielo. Also, criteria for the use of BP-G described at the FDA–CVM site of the USA, the EMEA agency of Europe, and SADER from Mexico and other regulatory agencies in Latin America are presented. The main pharmacological features of BP-G and the difference between benzathine, procaine, sodium, and potassium salts are presented. Also, analysis is made on the support of the use BP-G and its different salts in complying with the PK/PD ratios required for these antibiotic derivatives. Hence, pharmacokinetics, drug interactions, and withdrawal times found in databases are presented and compared with the commercial inserts available from products in the market. The legal status of BP-G and its derivative salts in Latin America and other countries is presented.
Results
BP-G is one of the most widely used antibacterial drugs in both human and veterinary medicine. It is therefore, a large market for the pharmaceutical industry, and various combinations with frail pharmacological support have been made available to veterinarians. The crystalline sodium and potassium salts of BP-G and the benzathine salt of BP-G have been incorporated into many existing preparations, arguing that higher and more prolonged concentrations are obtained with this combination. However, there is no evidence published on this issue. Yet, these preparations are presented as sustained-release ones, capable of achieving high and sustained therapeutic concentrations. Pharmacokinetic evidence does not support these claims for such combinations of BP-G derivatives, and it is unlikely that such combinations provide better serum profiles than those observed for procaine BP-G alone. Incorporating the BP-G benzathine in veterinary preparations has been banned in many countries. Nevertheless, in Latin America, such combinations exist, reducing the dose of procaine BP-G that should be injected; i.e., ≥ 20 000 IU/kg of body weight every 12-24 h. Within the framework of the rational use of antimicrobial drugs in veterinary medicine, advanced by the World Health Organization (WHO) and based on pharmacological considerations, it is proposed that combinations of BP-G derivatives be restricted, allowing only BP-G procaine alone preparations. Also, formulations of BP-G incorporating other active principles should be accepted if solid pharmacological support is presented. To date, combined preparations of BP-G and their derivatives have shown no formal pharmacological support. Based on the information presented in this review, it is feasible to conclude that the only BP-G derivative that should be available for veterinary medicine is procaine BP-G.
Conclusions
It is necessary to optimize the use of BP-G to limit the development of bacterial resistance. Injected procaine BP-G is the only penicillin derivative that meets the PK/PD ratios required for this time-dependent antibiotic. The recommended dose of procaine BP-G in different species should be ≥ 20,000 IU/kg every 12-24 h IM, and under this dose-scheme, a withdrawal period of 7 days is required. Unfortunately, some products on the market indicate lower doses and shorter withdrawal times. If the proper therapeutic result is expected and bacterial resistance is limited, it is essential to consider the PK/PD arguments presented here.

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1023 - Clinical evaluation of an alginate-enro-c (enrofloxacin hydrochloride/dihydrate) hydrogel for the treatment of various lesions and in various procedures in bovines.

Author: LILIA GUTIERREZ OLVERA

Objectives

A hydrogel based on alginate-sodium, combined with a patented new solvate of enrofloxacin (hydrochloride-dihydrate) (enro-C; Patent 472715: *Instituto Mexicano de la Protección Industrial, Mexico City*) was designed and manufactured at the Laboratory of Pharmacology of the Vet School (UNAM) under good manufacturing practices, and according to the following composition: 0.8% enro-C in 2% sodium alginate and 0.5% propylene glycol (Al-enro-C/HG). The gel was manufactured every month and poured into 80 mL plastic bottles, which were kept refrigerated at all times (4° C). Enro-C batches were prepared as indicated in the corresponding Patent. Based on previous experiences in other veterinary species [1, 2], various wound and potentially infectious sites and as part of different procedures were treated with this Al-enro-C/HG hydrogel, i.e., to prevent newborn calves' navels infections, as a wound healing ailment both in uncomplicated and in chronic - bacterially infected wounds, with or without necrotic-crusty skin, and also in wounds unsuccessfully treated with systemically or topically administered antibiotics on at least one occasion. In this study, the hydrogel was also utilized to hasten the healing of remaining stumps after dehorning of calves, and based on the biocompatibility of alginate and the almost neutral pH of enro-C, the hydrogel was also tested as a treatment of pink-eye (*Moraxella bovis*), teat cracks and fistulas, and in two cases of severe body-burning that occurred in a barn fire.

Material and methods

This study was approved by the Committee for Postgraduate Research, Care and Use of Experimental Animals, following Official Mexican Regulation NOM-062-ZOO-2001. Although most clinical cases were from the dairy industrial conglomerate at Tizayuca, Hidalgo State, Mexico, some were from other dairies. When possible, bacteriological analysis was carried out from swabs before the initial treatment. Neither antibacterial susceptibility nor genetic antibiotic-resistant patterns were attempted. Treatments were carried out only with Al-enro-C/HG by applying it directly in the targeted area (umbilical stump, affected eye, open wound, or the whole affected area in the burned cows), ensuring direct contact with the affected area. In abscesses, they are first cleaned vigorously, and their contents gently drained. Then, the hydrogel was inserted with a syringe, leaving a draining open wound. This was repeated at least two times a day and preferably up to four times daily in severely affected cases. On various occasions, the gel was inserted into an almost closed wound in which an abscess or a cavitated wound was forming. In these cases, an opening of the lesion was ensured to allow debridement. In almost all the studies, the experimental setting followed the following standard features: they were all field clinical cases of the different diseases or conditions, i.e., none of the diseases was induced; a reference or gold-standard treatment was included in all listed trials, and no untreated bovines were ever included as participant bovines belonged to various commercial farms and the ethics of such action was regarded as doubtful. For statistical analysis, clinical signs before treatment and after healing were compared using a Wilcoxon matched-pair rank test with Z approximation. Progression was recorded every three days, and ANOVA quadratic repeated assessment test was set for the three severity grades: $F = 38.23$; $P = 0.0001$ (The IBMSPSS package was used). In a particularly challenging case, three cows suffered severe burns after a burning roof collapsed on them. They were treated with fluids and electrolytes, as well as sedation (xylazine) and palliative care. However, the primary treatment was with the hydrogel. Two of the three cows recovered fully, and due to their suffering, it was considered that the third cow should have been sacrificed.

Results

Some of the clinical challenges to assessing the Al-enro-C/HG hydrogel are outlined. In preventing navel infections in newborn calves, it was observed that the umbilical stump involution was evident in the enro-C-treated animals on day one as most stumps were noticeably dried. Stump detachment occurred within an average of nearly 30 days, and the umbilical scars did not present infection in any (η=209). Conversely, in the calves treated with iodine-polyvinylpyrrolidone (η=205) stump drying was observable after 72 h, and it detached within an average of 32 days ($P < 0.05$). In stumps treated with the referred hydrogel, dirt stuck less, and the gel formed a protecting layer around the umbilical scar when the stump was either absent or too short. It was concluded that the hydrogel successfully allows rapid umbilical stump involution in newborn calves.

As far as the three burned cows are concerned, they were treated with fluids and electrolytes, as well as mild sedation (xylazine) and palliative care, i.e. administration of meloxicam. However, the primary treatment was with the hydrogel. Two of the three cows recovered *at Integrum,* and due to their suffering, it was considered humane that the third cow should be sacrificed. Representative images of the cases will be presented.

Another clinical setting was using the hydrogel to treat infectious bovine keratoconjunctivitis. The bacteriological efficacy was established and compared to an ophthalmic commercial florfenicol preparation. Results revealed similar potencies of the florfenicol and the enro-C hydrogel vs. *Moraxella bovis*. However, clinically, the animals (cows and calves) treated with the hydrogel (η= 61) presented a much faster progression and end-time to complete healing, as compared to what was observed in the florfenicol treated cattle (η= 58).

Other cases presented similar and statistically significant differences in favor of the hydrogel when tested against the control/reference group and evaluating weight gain and an analogic pain score assigned to calves. In 78 calves (η=39 calves per group) underwent surgical disbudding. Inflammation of the surrounding tissue and pain score was better for the hydrogel-treated calves ($p < 0.05$).

Leaving aside the peculiarities of each set of cases here presented, it is possible to conclude that Al-enro-C/HG hydrogel is highly effective and the improvement observed becomes rapidly evident, particularly when compared to other topical procedures to resolve the variety of cases listed ($P < 0.001$ in most instances). Its application is simple, painless and, from what could be perceived, even induces some relief and well-being in the treated animals. This product is postulated as an easy and highly effective way to treat various injuries and infections within the daily medicine of bovines.

Conclusions

In bovine daily practice, many wounds heal as part of the natural tissue restoration tendency. However, wounds are occasionally infected as bacteria access the particular tissue deeper. Treatment with antibacterial drugs locally, systemically, or both may resolve many cases. However, unresponsive, full-thickness-open wounds with significant tissue damage may turn wound healing into a long-lasting and challenging process that hinders bovine productivity. Wound dressings with local antiseptics or antibiotics are often applied, and their success is variable at best.

In contrast, a single product, the Al-enro-C/HG dressings, can be classified as passive, interactive, and bioactive based on their tissue vs. healing material interaction degree. In these trials, the outstanding healing capabilities of the hydrogel sufficed and needed no systemic antibacterial drug support. Although in several of the trials, a reference group with an accepted treatment was used, a control group was considered unethical in some cases, i.e., the burned cows or other animals whose injuries were so severe that endangered the animal's well-being, such as deep cavitated wounds with deficient blood supply, and presence of necrotic tissue.

Wound dressings based on alginate material are well-known in human medicine, and various commercial preparations are available for wound management. However, the enro-C-sodium alginate hydrogel utilized in this trial is unique as the chemical compatibility of sodium alginate

was only achieved with this new crystal solvate of enrofloxacin and not with standard enrofloxacin or its HCl derivative or any other fluoroquinolone tested. It is postulated that Al/enro-C/HG achieves a sustained release of enro-C that lasts three to six hours, depending on the applied site. This proposal has been reproduced in vitro but not in situ or in vivo, as the clinical nature of these trials was incompatible with the chemical determination of enro-C in samples of the affected or surrounding tissues. However, congruency of MIC vs. enro-C concentrations is foreseen, given the obtained results. One obvious advantage of the Al/enro-C hydrogel is that it acts as a natural hemostat. Also, the gel-forming property of alginate helps remove the dressing without much trauma, and often, this procedure was unnecessary. It became apparent to clinicians that the hydrogel tested reduced the pain the patients experienced, a feature already established for humans with other alginate-based dressings and evident in the dehorning of calves. Also, It has been shown that sodium alginate possesses outstanding biocompatibility properties, gel-forming ability, non-toxicity, and biodegradability. Furthermore, results suggest some form of tissue engineering is occurring and requires further research as it has been shown that alginates possess a potential for protein delivery in wounds that are being healed.

Detailed microbiological studies of susceptibility and bacterial resistance are needed to assume that in many of these cases the hydrogel could have delivered sufficient enrofloxacin to kill sensitive bacteria. Studies are currently being carried out to establish the relationship between tissue concentrations of enro-C and MICs patterns of various pathogens. For example, a well-diffusion analysis implemented for *Moraxella* spp. revealed high sensitivity to the hydrogel (MIC_{90} 0.03 µg/mL), and similarly for Trueperella pyogenes (MIC90 < 0.25 µg/mL) and for *Staphylococcus aureus* (MIC90 =0.25 µg/mL) .

As with other farm species, cattle suffer injuries and require special care for wounds and other conditions, i.e., pink-eye. Apart from the obvious recommendation of safeguarding these animals' envirionment, the Al-enro-C/HG presented in this study compilation shows promising results. Epidemiological consequences of its use must be studied before it is introduced into clinical practice.

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1281 - Effect of additional prednisolone in intramammary treatment of clinical mastitis

Author: J. M. Swinkels, G. Porcheron, O. Roy, M. Catalas, F. Leboeuf, D. Ledoux, A. De Boyer Des Roches

Objectives

Intramammary (IMM) antibiotic combined with prednisolone to treat clinical mastitis (CM) are popular where these products are registered, although field trials confirming the additional effect of IMM prednisolone are scarce. Because automated milking systems (AMS) are increasingly installed in the EU, our objective was to compare the effect of IMM prednisolone and cefapirin to cefapirin alone on the outcome of naturally occurring CM cases, using AMS installed milk and cow monitoring sensors.

Material and methods

Nine French dairy farms with HF cows in free stalls, selected on capabilities and willingness to participate, milking in a Lely A4 or A5 AMS, having HRLDn milk and cow sensors installed, were included. SCR milk sensors were used to collect data on milk production, milk color, temperature, SCC and conductivity at quarter level, and SCR cow monitoring sensors attached to the neck collar were used to monitor a 24h rolling average on rumination and activity.

CM was detected using the AMS Report 12 and 23 and/or a high conductivity and/or a high cell count in the T4C or Horizon software. The farmer was asked to check this alert at least twice a day, and confirm the mastitis clinically, before calling the local veterinarian for clinical examination, sampling milk for bacteriology and apply the first treatment within 2 hours.

Three subsequent treatments and behavior observations were performed by the farmer with 12h intervals. Treatment was randomly performed by either a commercially available tube (300 mg of cefapirin with 20 mg of prednisolone (**P**), Mastiplan LC, MSD Animal Health), or a tube with only 300 mg of cefapirin (no prednisolone: **NP**). The farmer and veterinarian were blinded to treatment. Randomization was performed with statistical software randomly determining the sequence of treatment P or NP.

Immediately before treatment and 14 ± 2 days later, the veterinarian took a milk sample for bacteriology. At each treatment, the farmer scored the CM case clinically and took a milk sample for cortisol measurement. Samples were frozen until collection to be sent to the laboratory. Milk and cow sensor data were collected at 14 days before and after the CM occurrence.

Results

Due to the early detection and farmers preference to defer from the treatment protocol in more severe (grade 3) CM cases, mostly mild (grade 1 and 2) CM cases were included. The study is still ongoing, results are preliminary, based on 73 of the intended 100 CM cases that showed a change of milk aspect, an increase of SCC and conductivity, drop of milk yield, and a decrease in activity and rumination. The most frequently isolated pathogens in group NP (n=35) and group P (n=36) were CNS; 34% and 25%, negative culture; 26% and 22%, Enterobacteriaceae, 3% and 28%, and *Streptococcus uberis*, 17% and 11%, respectively. Overall bacteriological cure rate was 77.3% and 82.6% in group NP and P, respectively, and not significantly different (Chi-square: $P = 0.6$).

Kaplan Meier survival estimate showed a significant difference ($P = 0.05$) in time to return to normal quarter SCC (< 200,000 cells/ml) in the P group. CM cases in the P group also had a significant effect on milk cortisol level ($P = 0.0001$) in milk during treatment. No difference was found in milk conductivity between the 2 groups due to a large standard deviation, and the statistical analysis of rumination, activity and milk yield showed only a numerical difference after treatment, being higher in the P group compared to the NP group. Full analysis, including also behavior observation data, will be available at the time of the WBC Congress.

Conclusions

Preliminary data show additional IMM prednisolone treatment of mild CM cases significantly reduced SCC and cortisol levels in milk during treatment, thereby improving milk quality and animal welfare. Both milk production, rumination and activity showed a numerical higher level during additional IMM prednisolone treatment.

1172 - Evaluation of the treatment efficacy of generic enrofloxacin compared to pioneer enrofloxacin for first treatment of naturally occurring bovine respiratory disease in a commercial feedlot

Author: Miles Theurer, Jessica Newberry , J. Trent Fox, Fabrice Payot

Objectives

The primary objective of this study was to compare the treatment efficacy of generic enrofloxacin [Tenotryl™ (enrofloxacin injection)] (TEN) to pioneer enrofloxacin [Baytril® 100 (enrofloxacin injection)] (BAY) for first treatment of naturally occurring bovine respiratory disease (BRD) on subsequent health outcomes in a commercial feedlot. The secondary objective was to evaluate health outcomes in dairy-beef cross cattle compared to traditional beef breed beef cattle along with treatment group administration.

Material and methods

The study was performed at Hy-Plains Feedyard located near Montezuma, Kansas, United States. Five hundred cattle identified with BRD by pen riders with rectal temperature were randomized to TEN or the BAY in a 1:1 ratio within each lot. Inclusion criteria for treatment study were pulled for BRD, rectal temperature 40°C, no previous treatments for disease, estimated > 60 days to harvest, clinical illness scores 1, 2, or 3, and absence of clinical signs of disease in other organ systems. Upon meeting inclusion criteria, cattle were randomized in a 1:1 ratio to 1 of 2 treatment groups within each lot: Tenotryl™ (TEN: 9.92 mg/kg body weight subcutaneous in the left neck) or Baytril® 100 (BAY; 9.92 mg/kg body weight subcutaneous in the left neck). Cattle treated for BRD were returned to their home pen and monitored for 60 days to observe subsequent health outcomes. A 3-day post-treatment interval was used for both the TEN and BAY treatment groups before cattle were eligible for retreatment. A gross necropsy was performed by a veterinarian or trained feedlot personnel on all enrolled animals which died during the study. A cause of death was determined for each case that died during the monitoring period based upon gross pathological lesions. Binary variables were created for treatment success and BRD case fatality risk. Treatment success was defined as not requiring additional treatment for BRD and not dying during the 60-day monitoring period. Case fatality risk was defined as cattle dying due to BRD during the 60-day monitoring period. Cattle were categorized by type (dairy-beef cross or traditional beef breed). Generalized linear mixed models were used for statistical analyses with distributions and standard link functions aligned with the outcome variable. Gaussian distribution was used for continuous outcomes (days on feed, body weight, and enrollment rectal temperature), binomial was used for dichotomous outcomes (treatment success and case fatality risk), and cumulative logistic was used for ordinal outcomes (sex, clinical illness score, and cattle type).

Results

There were no differences in first treatment success (64.29% vs 58.16%; $P = 0.19$) or case fatality risk (10.97% vs 10.65%; $P = 0.91$) comparing the TEN group to the BAY group respectively. Traditional beef breed cattle had greater body weight at time of enrollment (890.3 lb vs 749.1 lb; $P < 0.01$) and greater third treatment success (84.44% vs 41.67%; $P < 0.01$) compared to the dairy-beef cross cattle.

Conclusions

There were no differences in health outcomes in cattle administered Tenotryl™ compared to Baytril® 100 for first treatment of BRD in commercial feedlot cattle. From an efficacy perspective, practitioners should be able to use these products interchangeably based upon the results of the study. Additional research on general animal health outcomes of dairy-beef crosses is needed for the industry.

1054 - The effect of metaphylactic use of tildipirosin for the control of naturally occurring bovine respiratory disease on performance and profitability in high-risk feedlot cattle

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Objectives

The Bovine Respiratory Disease (BRD) complex is a multifactorial disease involving an interaction between environment, host (cattle) and agents (viruses and bacteria) and is the most prevalent disorder in feedlot cattle in Brazil. The biggest challenge of BRD is an early diagnosis and treatment. Metaphylaxis is the practice of administering an approved antimicrobial to an entire lot or pen of cattle with the intent of controlling the incidence of BRD in cattle at significant risk for BRD. The aim of the study was to evaluate the impact of metaphylaxis on morbidity, average daily gain (ADG), carcass weight, processing costs, revenue, and profits of high-risk cattle of BRD in feedlot.

Material and methods

A total of 208 high-risk Crossbred bulls (*Bos taurus × Bos indicus*), aged 24-30 months, from a commercial feedlot in Frutal, Minas Gerais, Brazil, were randomly assigned to two groups. At the time of processing, the Met-Group (n=104) received a single subcutaneous administration of tildipirosin (Zuprevo®, MSD Animal Health) at 4 mg/kg body weight (0.022 mL/Kg BW), while the Control-Group (n=104) received subcutaneous 0.9% saline at the same dose. Both groups were processed and received a vaccine for clostridial disease, a BRD vaccine, and oral anthelmintics. Each group was housed separately during the rainy season. The inclusion criteria for high-risk cattle were external source acquisition, a Body Condition Score (BCS) of 1 and 2 (scale from 1 to 5), a distance traveled >500 km and during >8 hours with three days of rest on arrival at the feedlot. All animals were weighed individually upon entry and upon loading for slaughter. Average daily gain was calculated for the period on feed. All animals were harvested, carcass data were collected, and the carcass weight was evaluated. All processing costs and values received for the carcass and the final profit of each animal were also calculated. Characterization of BRD was based on the DART system. Statistical analysis of the data was performed using the ANOVA test. Student's T test, was applied to the average daily gain and carcass weight, variables, with a 5% significance level using the R software.

Results

The average body weight upon entry of *Met-Group* and *Control-Group* were 360.8 kg and 361.8 kg, respectively. The average daily gain (mean ± SE) for the *Met-Group* was higher (1.82 ± 0.03 kg) than for the *Control-Group* (1.67 ± 0.03 kg), P=0.0012. The carcass weight (mean ± SE) was also higher for the *Met-Group* (288.93 ± 2.94 kg) vs the *Control-Group* (280.13 ± 2.94 kg); P=0.0035. Metaphylaxis increased the processing costs (R$46.52 vs R$9.42 for the *Met-Group* and *Control-Group*, respectively). However, these costs were compensated by increased revenues (3.14%) and profits (11.08%) of the Met-Group vs the Control-Group

The difference in performance and profitability between the two groups could be explained by a lower BRD morbidity rate in the *Met-Group* compared to the *Control-Group*, (8.65% vs 23.08%, respectively).

Conclusions

This study demonstrates the advantage of antimicrobial metaphylaxis with tildipirosin in high-risk cattle on their arrival at the feedlot, in reducing BRD morbidity and improve average daily gain, carcass weight and profitability.

Genetics

1683 - Seasonal sinusoidal modeling and deviations in milk traits due to short-term heat stress as indicators of the resistance and resilience of different dairy cow breeds on the plains and in the mountains

Author: Giovanni Bittante, Gustavo Javier Martinez Marin, Hugo Oswaldo Toledo Alvarado

Objectives
The objective of this study was to use the regression sinusoidal function for modeling and distinguish clearly the long-term (circannual) seasonal pattern from acute, short-term changes in relation to both climatic traits (daytime and air temperature) and milk production traits (daily yield, and fat and protein percentages).
Material and methods
We used 65,883 test-day records from 117 herds (105 on the plains and 12 in the hills and mountains) from the Veneto region (northeastern Italy), with records comprised daily milk yield (dMY, kg/d), and the milk fat (FAT%) and protein (PROT%) concentrations predicted by Fourier-transform infrared spectroscopy with a Milkoscan FT7 DC (FOSS Electric). The geographical coordinates and climatic characteristics of the study area were data from those 39 weather stations from which at least one participating. The meteorological data consisted of minimum daily temperature (T_{min}, °C), maximum daily temperature (T_{max}, °C), and average daily temperature (T_{mean}, °C). Daytime (DayTime, in h) was calculated for each weather station according to its latitude using the equation suggested by Allen et al., (1998). To estimate the parameters of the sinusoidal function (A, C and D), we used the PROC NLMIXED (SAS 9.4). As the sinusoidal function and month effects represent both the circannual variation of a trait, to obtain a sequential attribution of total variation to them, the type I ANOVA was obtained with a PROC GLM (SAS 9.4). The model used for temperature traits included: year, sinusoidal function, month, altitude class of weather stations, the interaction altitude with sinusoidal function, weather stations, interactions of weather station with year and month. The DayTime data were analyzed with a simplified model derived from the previous model with the deletion of the year and the interaction with weather stations. The model for analyzing milk production traits was derived from the model for meteorological traits, but with weather stations substituted by Herd, and with the addition of the sources of variation due to individual cows: parity, lactation stage, breed and its interaction with sinusoidal function and altitude.
Results
The sinusoidal circannual function was tested on daytime and in temperatures, where they represented almost all the variation (99% and ≥ 93.0% respectively). The effects of the class of altitude of the weather stations (plains vs hills and mountains) accounted for 1.71% to 2.48% of model explained variation, individual weather stations within altitude 3.47% to 1.01% and other factors represented <1% or were not significant. As expected, the most important sources of variation were herd and stage of lactation, while parity was important only for milk yield. Among the calendar traits, the long-term sinusoidal function resulted more important than the year and the short-term monthly deviations. The milk fat and protein percentages reached their zenith values in December-January, and their nadir values in June-July. The pattern was opposite to that of daytime and was about one month earlier than that of temperature. This seems to indicate that milk quality is affected by photoperiod (daytime) more than by temperature, which is confirmed by the non-significant interaction of altitude with the sinusoidal function for these traits (the plains and mountains have the same daytime, but different temperatures). The

sinusoidal function of milk yield, on the other hand, is delayed by about three months compared with daytime, and by two months compared with temperature (nadir values in September-October), probably because of the depletion of body reserves due to reduced feed intake in hot periods. The class of altitude affected the amplitude of the sinusoidal function, reflecting the greater level of climatic stress experienced on farms in the humid, subtropical climate of the plains compared with the oceanic climate of the hills and mountains. Significant decreases in milk yield, especially between June and July, compared with the sinusoidal function were interpreted as short-term deviations due to acute heat stress.

Conclusions

In conclusion, the sinusoidal function was found to describe almost all (DayTime) or most (air temperature traits) long-term (circannual) climatological variations. In the case of milk traits, the circannual sinusoidal function was also the largest calendar source of variation (much greater than the effects of year or short-term monthly deviations from the sinusoidal function). The Alpine breeds appear to be more resistant (lower amplitude) and resilient (delayed response) to heat stress than Holsteins and crossbreds.

1547 - Quantile regression for prediction of complex traits in braunvieh cattle using SNP markers and pedigree

Author: Jonathan Emanuel Valerio Hernández, Paulino Pérez Rodríguez, Agustín Ruíz Flores

Objectives

The objectives of this work were to study the predictive ability of quantile regression (QR) models using SNP marker information with and without pedigree information and compare them to genomic best linear unbiased prediction (GBLUP) and single-step GBLUP methodologies, for predicting complex traits such as birth, weaning and yearling weights in Braunvieh cattle. The QR models considered were: QR with only SNP markers (QRM), and QR with SNP markers and pedigree (QRH). The predictive performance of the QR, GBLUP and single-step GBLUP models was evaluated using both real and simulated data with different degrees of asymmetry and proportions of atypical phenotypes, through cross-validation.

Material and methods

Genotypes

Genotypes were obtained for 300 animals including 236 females and 64 males born between 2001-2016 from 5 herds located in eastern, central, and western Mexico. Hair samples were collected for genotyping using the GeneSeek Genomic Profiler Bovine LDv.4 Chip with 30K and 50K SNP markers, with 150 animals genotyped with each chip. SNPs common to both chips (12,835) were used. After quality control, 9,628 SNPs were retained for further analysis.

Phenotypes

Phenotypic and pedigree information for the Braunvieh cattle population was obtained from the Mexican Braunvieh Association database. Birth weight (BW), weaning weight (WW), and yearling weight (YW) records were used for analysis. Contemporary groups (CG) were defined by combining effects of herd, year, season, and management group. After editing, 330 BW, 267 WW, and 232 YW records remained.

Models

The quantile regression with markers only (QRM), markers and pedigree (QRH), genomic best linear unbiased prediction (GBLUP) and single-step genomic best linear unbiased prediction (ssGBLUP), models were used.

- QRM: Uses quantile regression to predict genetic values from SNP marker information. Allows modeling asymmetric distributions of phenotypes.
- GBLUP: Performs genomic prediction through best linear unbiased prediction assuming normal distribution of errors. Uses only SNP marker information.
- QRH: Combines SNP marker and pedigree information into a single quantile regression model.
- ssGBLUP: Extends GBLUP by incorporating pedigree information in addition to SNP markers into the relationship matrix. Assumes normality.

Cross-Validation

5-fold cross-validation was performed, with 80% of data for training and 20% for validation. Correlations between observed and predicted values were averaged across folds.

Simulation

Data was simulated to compare QR and GBLUP under asymmetric distributions (0.950, 0.975, 0.999) with outliers (5% and 10%). Phenotypes were generated from a skewed normal

distribution with varying asymmetry and outlier proportions. Predictive performance was evaluated through correlations, mean squared error, residual variance, and deviance information criterion.

Results

Real Data

In the analysis of real data, quantile regression (QR) exhibited higher correlations between observed and predicted phenotypes for birth weight (PN), weaning weight (PD), and yearling weight (PA) in Braunvieh cattle, compared to Genomic Best Linear Unbiased Prediction (GBLUP) and single-step GBLUP (ssGBLUP). The Mean Squared Error (MSE) values were generally lower for QRM (QR with markers) and QRH (QR with markers and pedigree) in weaning and yearling weights, while GBLUP and ssGBLUP performed better for birth weight. The components of variance associated with the error obtained with QRM and QRH were lower than those obtained with GBLUP and ssGBLUP. Lower Deviance Information Criterion (DIC) values were generally obtained with QRM θ=0.75 and QRH θ=0.75, except for birth weight with the marker-only scenario, where the lowest DIC was obtained with QRM θ=0.25.

Simulated Data

In the simulation experiment with asymmetric data and the presence of outliers, QR demonstrated superior performance compared to GBLUP. Correlations between true marker effects and estimated effects, as well as correlations between true signals and estimated signals, were consistently higher for QR across different levels of asymmetry and proportions of outliers. The MSE values, estimates of variance components associated with the error, and DIC values were generally lower for QR θ=0.75 in the simulation study.

Conclusions

In real data, QR showed higher predictive correlations for WW and YW, while GBLUP and ssGBLUP were slightly better for BW. In simulation, QR had higher correlations between true and estimated marker effects and signals compared to GBLUP, especially with more asymmetry and outliers. QR also had lower residual variance and DIC, indicating better fit. Overall, QR performed equal or better than GBLUP and ssGBLUP for growth trait prediction. Advantages of QR were more noticeable with asymmetric and outlying data.

1542 - Genetic parameters of milk composition of Brown Swiss dairy cattle in Italy.

Author: Eunice Zurizadai Flores Ramírez, Hugo Oswaldo Toledo Alvarado

Objectives

Estimate genetic parameters: heritability, and genetic correlations, of milk yield, and milk composition: fat, protein, lactose, monounsaturated fatty acid, polyunsaturated fatty acid, saturated fatty acid, unsaturated fatty acid, short chain fatty acids, medium chain fatty acids, and large chain fatty acids, of Brown Swiss dairy cows from Northern Italy.

Material and methods

A database with 531,504 test day records of 2,218 herds, registered from 2010 to 2017 was used. The fixed effects used in the genetic parameter estimation were the year-season effect (two seasons each year), number of parity (1, 2, 3), and days in milk category (11 categories with 30 days on each). The pedigree used had 93,721 sires, 93,803 dams, and 104,340 labels.

The model used was:

a. $=Xb+Ha+Zu+Wpe+e$

where **y** is a vector of observations of each phenotype (milk yield, fat, protein, lactose, monounsaturated fatty acid, polyunsaturated fatty acid, saturated fatty acid, unsaturated fatty acid, short chain fatty acids, medium chain fatty acids, and large chain fatty acids); **b** is a vector of fixed effects (parity, days in milk, year-season); **a** is a vector of random effects of the herd; **u** is a vector of random effects of the animal; **pe** is a vector of permanent environment random effects; **e** is a vector of random residual effects; **X**, **H**, **Z** y **W** are incidence matrices relating the records to the respective effects. The same model was used in bivariate analysis combining two traits at the time to estimate genetic correlations.

Results

The results obtained for the heritabilities estimates were similar to previous reports in other populations or breeds. The heritability for milk yield was 0.12 ±0.004 similar to previous estimations of 0.10 (Penasa et al., 2015) and 0.41 (Schopen et al., 2009). The heritability of fat was 0.22 ± 0.005 which is similar to the estimates by Visentin et al., (2019) of 0.25 and Penasa et al., (2015), of 0.2. The estimations of the heritability for protein have a range between 0.26 (Penasa et al., 2015) to 0.66 (Schopen et al., 2009), our estimation was 0.30 ± 0.007. The heritability estimated for lactose was 0.33 +-0.007 lower than the reported by Cost et. al (2019) of 0,43 in Holstein cattle. The heritabilities for fatty acids groups were: monounsaturated 0.17 ± 0.006 higher than reported by Penasa et al., (2015) of 0.08, polyunsaturated 0.16 ± 0.006 higher than 0.08 reported by Penasa et al., (2015), saturated 0.12 ±0.005 lower than 0.25 reported by Penasa et al., (2015), and unsaturated 0.15 ± 0.005 higher than reported by Penasa et al., (2015) of 0.07. According to the length of the chain of the fatty acids, the heritabilities were: short-chain 0.27 ± 0.008, medium-chain 0.26 ± 0.007 lower than reported by Stoop et al., (2008) of 0.49 and long-chain 0.12 ± 0.005 similar of 0.11 reported by Stoop et al., (2008).

Genetic correlations: The lowest genetic correlations were estimated for the saturated fatty acids and unsaturated fatty acids (-0.92± 0.001), and for saturated fatty acids and long-chain fatty acids (-0.80 ± 0.01). The highest genetic correlation was estimated between the long-chain fatty acids with the monounsaturated fatty acids (0.84 ± 0.01) and between the long-chain fatty acids with the unsaturated fatty acids (0.81 ± 0.01).

Conclusions

The heritability estimates are similar to the ones reported in other breeds or production systems. The heritability depends on the breed, the production system, and the country. The highest heritability was lactose followed by protein and short-chain fatty acids. There is a high absolute genetic correlation between different phenotypes, therefore it is possible to make indirect selection indices to improve the milk composition in Brown Swiss dairy cows.

1458 - Recessively inherited osteochondrodysplasia TGDS and LAMA4-related in Chianina cattle

Author: Joana G.P. Jacinto, Tolulope G. Ogundipe, Cinzia Benazzi, Irene M. Häfliger, Luisa V. Muscatello, Marilena Bolcato, Arcangelo Gentile, Cord Drögemüller

Objectives
Skeletal dysplasia encompasses a heterogenous group of genetic disorders characterized by an abnormal development of bones, joints, and cartilage. Two Chianina calves with congenital skeletal malformations were identified. The aim of this study was to report a bigenic recessively inherited form of osteochondrodysplasia (OC) in Chianina cattle. Herewith the clinicopathological phenotype of affected animals and the molecular basis are presented. Moreover, the prevalence of the deleterious alleles in the Chianina population are estimated.
Material and methods
This study deals with a total of 358 Chianina cattle, including two OC-affected calves, their dams and sire and, finally 353 healthy control Chianina bulls. The affected animals underwent a clinicopathological investigation. Genomic DNA was obtained from the OC-affected animals (ear cartilage tissue samples), their respective dams (EDTA blood samples) and the common sire (semen) using standard methods. Furthermore, genomic DNA was also obtained from semen of 113 Chianina AI top sires' semen as well as from blood samples of 237 Chianina young bull calves shortlisted for admission to the performance test at the testing station in the years of 2017, 2018, 2019 and 2020. Whole genome sequencing trio-approach was performed. The sequenced reads were mapped to the ARS-UCD1.2 reference genome and single-nucleotide and small indel variants were called. In order to identify private variants, the genotypes of the OC cases were compared with a global cohort of 5347 cattle genomes of various breeds. In silico tools were used to predict the biological consequences of the detected variants. Candidate variants were visually inspected. A PCR-based direct gene test was applied to assess the frequency of the identified mutant alleles in the Chianina bulls.
Results
The cases presented retarded growth, poor nutritional status associated with muscular atrophy and angular deformities of the hindlimbs. Radiologic examination revealed generalized osteopenia and shortening of the limb long bones. Post-mortem examination showed chondrodysplastic limbs and dilation of the heart right ventricle. Histological examination of the physeal cartilages were characterized by multifocal mild to moderate loss of columnar arrangement of chondrocytes. Osteopenia was also observed. Genetic analysis identified a deleterious homozygous missense variant in *TGDS* (p.Tyr54Asn) and a splice-site variant in *LAMA4* with both uniquely present in the cases. Parents were heterozygous and overall allelic frequency in the male Chianina population for the *TGDS* variant was 5% and for the *LAMA4* variant was 2%.
Conclusions
In our study, a comprehensive clinical, histologic, and genetic evaluation of Chianina calves with OC was performed. We identified two homozygous most likely disease-causing variants affecting *TGDS* and *LAMA4.* After gene function analysis, taking into account the occurrence of the variant alleles in a global control cohort, the rarity and breed specificity of the variants, and in silico effect predictions, only the homozygous variants affecting *TGDS* and *LAMA4* were considered the most likely genetic cause of the observed phenotype. This study notifies Chianina veterinarians and breeders about the potential emergence of OC in the future. Despite the relatively low prevalence of the pathogenic TGDS and LAMA4 alleles, it emphasizes the importance of genetic testing as a preventive measure to avoid the unintentional occurrence of more affected cattle.

1440 - BLIRD, a new genetic disorder affecting immunity and life expectancy in Holstein dairy cattle

Author: Gilles Foucras, Lucie Dutheil, Florian Besnard, Guillaume Tabouret, Blandine Gausseres, Laurence Guzylack-piriou, Aurelien Capitan

Objectives
As a result of intensive genetic selection, bovine dairy breeds show high consanguinity trends. This leads to an increase in health problems, a reduction in life expectancy with an elevated juvenile mortality rate. Searching for enrichment or depletion in homozygous haplotypes (HHED mapping) in groups of animals with different life trajectories led to the discovery of several new recessive genetic defects affecting longevity in French Holstein cattle. The present study describes the traits and the pathophysiology related to the p.G375S ITGB7 point mutation.
Material and methods
To characterize the impact of the point mutation on bovine health, clinical signs and associated lesions were recorded in a series of cases and control animals that were genotyped for this mutation as part of routine genomic evaluation. During the epidemiological study, a total of 25 homozygous mutant females were studied with in-herd-matched wild-type controls. Phenotypic traits (weight, size) and health conditions were recorded. Blood and faecal samples were collected to describe the biological consequences of the point mutation (haematology, biochemistry, parasitology, faecal microbiota, biochemistry, …). Then, based on the basic knowledge of the protein function, we carried out a case/control study in a small number of cattle to further investigate the differences between homozygous mutant and wild-type, and to determine altered functions at the organ and system levels. Post-mortem examination was also undertaken for identification of gross lesions and microscopic examination was done on various tissue samples. Analysis of the immune cell composition of blood, lymph nodes and intestine wall by single-cell RNA-sequencing, in a comparison between carriers and non-carriers, helps describe the type and extent of the anomalies related to the point mutation, that were confirmed by flow cytometry.
Results
The lifespan of homozygous mutants was significantly reduced, and two periods of heightened mortality rate were identified (exponential growth phase, and early lactation). On average, affected animals were shorter, lighter and had lower performances (such as ADG or milk yield). On the other hand, most biochemical parameters were not affected by the mutation, but a marked lymphocytic leucocytosis and moderate eosinophilia were identified that may serve as phenotypic traits to identify carriers in the non-genotyped population. We confirmed the depletion of β7integrin-expressing CD4 T cells in the lamina propria of the jejunum, confirming the defect of CD4 T lymphocyte homing and retention in the digestive tract area due to ITGB7 altered functions. In the blood, a high proportion of CD4 T lymphocytes with a memory/activated phenotype was enumerated while B lymphocyte proliferation was noticed. Histopathology of the lymphoreticular system indicated a disturbed immune system not only in the mesenteric but also in the peripheral lymph nodes, with intense follicular hyperplasia.
Conclusions
All these observations are aligned with the description of a new genetic disorder we named BLIRD for Bovine Lymphocyte Intestinal Retention Default. We show that this inherited mutation leads to ill-thrift and reduced lifespan in Holstein dairy cattle. Non-pathognomonic symptoms may be confused with those of common diseases of environmental origin, but the haematological profile is sufficient to detect suspected carriers.

1472 - DYRK1B haploinsufficiency in Holstein cattle with epilepsy

Author: Joana G.P. Jacinto, Marilena Bolcato, Irene M. Häfliger, Arcangelo Gentile, Cord Drögemüller

Objectives

Epilepsy is a complex group of neurological disorders characterized by spontaneous recurrent seizures. It can be classified into idiopathic (genetic) epilepsy and structural. Furthermore, the seizure types can be classified according to their semiology as focal epileptic seizures, generalized seizures or focal epileptic seizures evolving into generalized epileptic seizures. The aim of this study was to report a dominantly inherited form of epilepsy in a Holstein heifer. Herewith the clinicopathological phenotype of the affected animal and the molecular basis are presented.

Material and methods

In this study, a Holstein heifer was referred to the Clinic for Ruminants of the University of Bologna at 6 months of age, weighing 125kg, with a history of multiple seizures since birth. The parents were reported to be healthy. The affected animal underwent a clinicopathological investigation. Genomic DNA was obtained from the affected animal (EDTA blood sample) using standard methods. Whole genome sequencing trio-approach was performed. The sequenced reads were mapped to the ARS-UCD1.2 reference genome and single-nucleotide and small indel variants were called. In order to identify private variants, the genotype of the affected heifer was compared with a global cohort of 5347 cattle genomes of various breeds, including 1204 Holstein. In silico tools were used to predict the biological consequences of the detected variants. Candidate variants were visually inspected.

Results

An epilepsy with focal seizures progressing to generalized seizures was diagnosed in a 6-month-old Holstein heifer. The seizures were characterized by brief pre-ictal phase with depression and vocalization. During ictal phase eyelid spasms, tongue contractions, nodding and abundant salivation were observed, rapidly followed by a convulsive phase with bilateral tonic, clonic or tonic-clonic activity and loss of consciousness. Finally, during the postictal phase the heifer was obtunded and disorientated, enable to perceive obstacles, hypermetric and with the tendence of pressing the head against the wall. In the inter-seizure phase, the heifer was clinically normal. Neuropathology revealed axonal degeneration in the brainstem and diffuse astrocytic hypertrophic gliosis. Whole genome sequencing of the affected heifer identified a private heterozygous splice-site variant in *DYRK1B* (NM_001081515.1: c.-101-1G>A), most likely resulting in haploinsufficiency due to loss-of-function. This represents a report of a *DYRK1B*-associated disease in cattle and adds *DYRK1B* to the candidate genes for epilepsy.

Conclusions

Our report describes the clinical, pathological and genetic findings in a Holstein heifer with idiopathic epilepsy with focal seizures progressing to generalized seizures. We propose here the first candidate causal *DYRK1B* variant associated with epilepsy. Our study highlights that the genetics of spontaneously occurring disorders in cattle is a valuable translational model system. Further functional studies into DYRK1B's role in epilepsies are needed.

Herd health and production medicine.

1431 - Usefulness of lung ultrasound (TUS) in the early detection of the bovine respiratory complex - experiences and results in Uruguay and México

Author: Omar Bellenda, Beatriz Almeida, Maria Jesús Crucci, Luis Albornoz, Stephanie Lara, Gabriel Ruiz, Jessica Rojo

Objectives

The Bovine Respiratory Complex (BRC) is a multifactorial pathology of great importance in dairy farms and intensive meat production (feedlots). Although clinical monitoring and control of some symptoms are helpful, the ultrasonographic study of some thoracic regions (TUS) in calves and young cattle, has shown greater objectivity and earlier diagnosis.

After developing the technique in Uruguay (2019-2020), different studies were carried out in some dairy farms in Southern Uruguay, with the aim of determining the prevalence of clinical and subclinical pneumonia in breeding calves.

Sometime later (2022-2023), we were spreading this technique in Mexico, training colleagues and specially, in a dairy cooperative at Querétaro. Recently, we have been collaborating, collecting and sharing data from that region, to try to obtain information on the BRC situation in that country, to compare and discuss the results.

Material and methods

The study in Uruguay was carried out in three dairy farms located in the South and quite far from each other. The initial population was dairy calves, older than 4 weeks and prior to weaning, performing a clinical examination based on the pneumonia signs score described by McGuirk (2008) and ultrasonographic diagnosis on both sides of the thorax (TUS). Images were stored and injury types were classified according to the scoring system detailed in Adams and Buczinski (2016). The animals with lesions were mainly treated with a mixture of Tilmicosin and Flunixin meglumine or in some cases, the separate administration of Florfenicol and Flunixin meglumine. Treated calves were monitored again within 7-10 days to evaluate response to treatment. The response was considered positive when a reduction in the size of the consolidation focusses or the absence of lung lesions was detected, and a negative response when there were no changes or the lung lesion worsened. The prevalence of pneumonia was estimated in the three farms and the prognosis after treatment was defined based on the clinical and ultrasound evolution.

Basically, the work model used in Uruguay was repeated in Mexico, although sampling could be carried out to determine the prevalent pathogens, and the spectrum of antibiotics was greater, including Enrofloxacin, Marbofloxacin and Tulathromycin, plus Flunixin meglumine.

Results

The target and reviewed population in Uruguay were 355 calves, of which 46 affected calves (13%) were treated with antibiotics and anti-inflammatory drugs, given that they presented clinical signs and/or lung lesions compatible with pneumonia, detected by TUS. The treatment response was evaluated based on the evolution of the animals. The prevalence of BRC, where clinical and subclinical sick animals are considered, was 10.4%, 39.4% and 10.1% for Farms No. 1, 2 and 3, respectively. It is important to highlight that 63.1% of the treated calves were asymptomatic, which reaffirms the importance of the use of this imaging technology. We found that 56.5% of the treated calves improved after the application of a single dose and only some of them (8.7%), needed a second dose.

In México, the data obtained, where studies were carried out in 295 calves, including 2 dairy farms in the State of Querétaro and other farm in the State of México, also show an incidence of respiratory disease: Farm No.1 had 47,3% of animals with symptoms, but in addition, 12.5% of asymptomatic animals, were found by TUS (subclinical disease). In Farm No. 2 with 25,3% of

clinical BRC and 8,4 % subclinical, and in Farm No. 3, 17.4% of calves were found with symptoms and injuries, and 26.6% were subclinical animals (detected by TUS). Animals with visible TUS lesions were treated, and a 61.5% improvement was found after the first injection, and 7.3% needed a second dose.

Conclusions

The observation of lung lesions through ultrasound in those animals without the presence of clinical signs allows for early detection and treatment of asymptomatic animals, with the consequent better evolution and prognosis.

It is concluded that the clinical evaluation of animals combined with the use of lung ultrasonography can be successfully used for the early diagnosis of pneumonia in calves and to evaluate the response to treatment.

1230 - Characteristics of fatal Mannheimia haemolytica fibrinous pleuropneumonia outbreaks on dairy farms in the Netherlands.

Author: Jasper het Lam , Irene Bisschop, Erik van Engelen, Evert van Garderen, Ynte Schukken

Objectives

Introduction

During the last decade, dairy farming in the Netherlands has been confronted with outbreaks of acute fibrinous pleuropneumonia, causing high-yielding cows to die in a short period of time. Thanks to the monitoring system in the Netherlands, in which pathological examination of deceased cows is a standard part, it appeared that the pleuro-pneumonia in these cows was associated with a pure culture of *Mannheimia hemolytica* (*Mh*) infections, and no other primary bovine pathogens were found in the affected lung tissues (Biesheuvel et al., 2021). Bovine respiratory disease (BRD) is known as a major cause of morbidity, mortality and antimicrobial use worldwide, albeit mostly in young cattle. BRD is generally considered to be induced by virus infections in combination with environmental stress, originating from suboptimal housing, feeding or transporting of the animals. As a consequence of these triggers, subsequent bacterial infections may lead to a fatal disease course. One of the most frequently isolated and most damaging respiratory infections is *Mh* (*(Klima et al., 2014)*. However, reports about *Mh* in adult cattle are scarce (Dorso et al., 2021; Gorden and Plummer, 2010). From 2004 to 2018 the percentage of fatal *Mh* infections in dairy cows submitted for pathology in the Netherlands increased significantly (Biesheuvel et al., 2021). Therefore, the aim of this study was to gain more insight in the circumstances leading to these *Mh* cases.

Material and methods

Material & Methods

Data was collected at animal and herd level from 50 farms that submitted at least one dairy cow for pathology with the diagnosis of fatal fibrinous pleuropneumonia caused by *Mh*. From a minimum of two cows with *Mh* symptoms it was defined as an outbreak. Farmers were asked to cooperate in a telephone survey of 45-60 minutes with around 90 questions. These questions partly concerned characteristics at animal level and partly at herd health management level. Further questions were about specific stressful incidents and presence of neighbouring herds with *Mh* outbreaks. Survey data were anonymously combined with other data sources concerning animal health statuses, laboratory results, identification & registration of animals and registration of antibiotic usage at farm level. Moreover, weather conditions previously to te onset of the outbreak were added to the datafile.

Results

Results and Conclusions

Most strikingly, while *Mh* is considered to be a secondary infection and one would expect such infections to be correlated to the known vulnerable fresh cow period, this was not the case. The cows were on average 213 days in milk (min. 40 - max. 535), had a good or high body condition score and a milk production level that was not significantly different from the herd average. At herd level, the median duration of an outbreak was 23 days (min. 1 - max. 499). The mean morbidity and mortality was 7,5% and 2,3% respectively, with a maximum mortality of 4,7%. The herds had an average milk production per cow close to the national Dutch average. However, the herds were bigger, delivered more milk per acre, had more cows per feeding place and more often introduced animals compared to average Dutch dairy farms.

In the acute phase the symptoms were nonspecific, which probably was the reason the first diseased cow on a farm was often recognised too late or had no diagnosis of a respiratory infection. These cows had a survival of 22%, while almost all the other cows once they were quickly and correctly diagnosed and properly treated. The identified *Mh* outbreaks most often occurred in the winter season. Moreover, on most farms the wind force and amount of rainfall

was high in the week previous to the start of an outbreak. Correlations that obvious stress factors such as ration changes, vaccinations, or previous diseases could not be demonstrated. Similarly, there were no correlations with BRD in young stock on these farms.

Conclusions

With this study we aimed to describe the characteristics of *Mh* outbreaks on dairy farms. This study does not provide evidence of specific risk factors associated with *Mh* outbreaks. More research is needed to find the causes why *Mh* outbreaks in adult dairy cows were historically scarce, and recently increased to approximately 50 outbreaks annually on apparently healthy dairy herds in the Netherlands.

1360 - Comparative Evaluation of Three Tests for Failure of Passive Transfer and Association with BRD and NCD Pathogen-Specific Antibodies in Neonatal Calves

Author: Egon Thesing, Bart Sustronck, Geert Vertenten

Objectives

Testing Failure of Passive Transfer (FPT) involves assessing the concentration of immunoglobulins (IgG) in the calf's blood serum. The most common method for diagnosing FPT is to measure the BRIX % using a refractometer. This measurement provides an indirect estimation of IgG levels.

An ideal method for FPT testing is to directly measure the IgG concentration using a quantitative enzyme-linked immunosorbent assay (ELISA) or radial immunodiffusion (RID) test. These tests provide more accurate results and a definite diagnosis of FPT. A serum IgG concentration of less than 10 mg/mL is generally considered indicative of FPT.

The objective of this study was to compare the results of three different tests for the evaluation of FPT with the serological status of various pathogen-specific antibodies in the neonatal calf.

Material and methods

This field study was performed on five different German dairy farms that are not vaccinating against Bovine Respiratory Disease (BRD) and Neonatal Calf Diarrhea (NCD) and with at least 100 lactating cows. Neonatal calves (n=106) aged 2-7 days were enrolled. All calves had received at least 2L of colostrum in the first 24 h of life. The IgG concentration in the serum was evaluated with three different methods: 1) Sandwich ELISA (Department of Veterinary Science, Munich) – FPT cut-off value: <10g/l; 2) ELISA-IgG (BIO-X, Rochefort) – FPT cut off value <10g/l; 3) Digital brix refractometry - FPT cut-off value: <8.3%. Furthermore, antibody levels against the following BRD and NCD pathogens were determined (Center for Diagnostic solutions, Boxmeer) : Bovine Corona Virus (BCoV), Bovine Rota Virus(BRV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 virus (PI3), *Mannheimia haemolytica (Mh), Mycoplasma bovis* (Mb) and *Pasteurella multocida* (Pm). The 3 diagnostic methods were compared using simple linear regression and correlation (Brix vs ELISAs), Blant-Altman plots (ELISAs) and a Bayesian latent class analysis was used to evaluate the FPT of the 3 tests. Finally mixed ordinal logistic regressions were used to evaluate the association between the IgG serum concentration and the pathogen specific antibodies in serum. The p-value was set at 0.05.

Results

The correlation between the Brix% and the IgG ELISA-Munich (R=0.71, p<0.001) is stronger than the correlation between Brix and the IgG ELISA-BioX (R=0.55, p<0.001). There was a strong agreement between the two ELISA methods as only two points fall out of the 95% Confidence Interval. A bias of 1.4 was identified which means that the ELISA-Munich on average results in an IgG serum concentration which is 1.4 g/l higher than the ELISA-BioX. This difference is significant (paired t-test p=0.048). So, we found a systematic, but not relevant bias in the difference in serum IgG concentration between the IgG-Munich and the IgG-BioX method. The limits of agreement were -12.5 and 15.34. This spread is small enough to suggest a fair agreement between the two ELISA tests.

Prevalence of FPT was estimated at 21%. The three tests have comparable sensitivity (±75%). The specificity of the two ELISA's is high (96%), however the specificity of the Brix method is lower (67%) resulting in a low positive predictive value (Ppv: if the test indicates FPT how sure can you be that the result is correct) of only 38%.

More important is the negative predictive value of the test when considering FPT. In other words, how sure can you be that a calf does **not** have FPT when the test indicates good transfer of immunity. In this case all three test are comparable (93%).

The overall accuracy of the two ELISA tests is comparable (92%). In this study the overall accuracy of the Brix method is considerably lower (69%).

Finally, looking at the association between the IgG serum concentration and the pathogen specific antibodies in the serum, it can be concluded that the higher the total transfer of passive immunity is, the higher the pathogen specific antibodies are.

Conclusions

The study suggests that the IgG ELISA-Munich and IgG ELISA-BioX methods have comparable performance in detecting FPT, while the Brix method showed lower accuracy. In addition, there is a strong association between passive immunity transfer and pathogen-specific antibodies which highlights the importance of adequate passive transfer for calf health and immunity.

1624 - Comparison of accuracy of sonography, rectal palpation, and PAG test for pregnancy diagnosis in cows

Author: Maria Sady, Zdzisław Gajewski, Noelita Melo de Sousa, Karolina Ferenc, Romuald Zabielski, Ewa Sady, Axel Wehrend, Jean-FRancois Beckers

Objectives
The accuracy of pregnancy diagnosis methods in cows can vary, and different methods have their strengths and limitations. Here's a brief comparison of sonography (ultrasound), rectal palpation, and pregnancy-associated glycoproteins (PAG) tests. Sonography is considered highly accurate for pregnancy diagnosis in cows. It allows visualization of the developing fetus and its heartbeat as early as 28-30 days post-breeding. Pregnancy-Associated Glycoproteins (PAG). Accuracy: PAG tests, are generally accurate for pregnancy detection but may not provide as much information as ultrasound. The aim of the present study was to use a new developed method for evaluating the PAG in blood and compare this results with the USG and rectal investigation for pregnancy diagnosis in the cows

Material and methods
Holstein-Friesian were 4 to 7 years old were used. The experiment was carried out on a group of 180 cows. All animals underwent pregnancy testing: ultrasonography with a real-time, B-mode diagnostic ultrasound Scanner (Esaote, Italy) equipped with a 5.0 MHz linear-array rectal transducer, 30-55 days after AI and rectal control after the 55th day of pregnancy. Blood were used for PAG tests. The concentration of bPAG was measured in serum. Blood samples were taken from jugular vein (v. jugularis) and stored at -20°C.The concentration of bPAG were measured in plasma. RIA method was used in plasma tests. The antibodies were produced after immunization of rabbits with purified pregnancy associated protein extract from cow placenta (bPAG 67kDa. The comparison of accuracy of those three methods for pregnancy diagnosis was established by using following factors: sensitivity and specify of method, calving records, and total accuracy.

Results
The profile of bPAG concentration in blood increased after 26-28 day of pregnancy**.** Average bPAG concentration in blood plasma for cows 18-42 days after AI varied from 1,20 ng/ml on the 20th day to 3,57 ng/ml on the 44th day. The profile of PAG concentration in the blood increased in second month of pregnancy and showed a rapid increase near the parturition. In the time between 18 till 44 day after A.I. the avarage concentration of bPAG was 3,7 ng/ml and varied from 1,30 ng/ml to 3,47 ng/ml. Variation analysis with the reliability level of 99% indicates a statistically significant correlation between progress of pregnancy and bPAG concentrations in blood (p value < 0,1). The positive predictive value between 18-60 days after AI was: for rectal palpation 85.7, for USG 86.4 PAG 80.9 and after 60 days post AI the ppv was respectively 98.1, 100 and 95.9. USG is a accurate way to diagnose pregnancy. We didn't find a strong correlation between pregnancy status and PAG concentration.

Conclusions
In summary, ultrasound is often considered the gold standard for pregnancy diagnosis in cows due to its high accuracy and ability to provide detailed information about the developing fetus. Rectal palpation is a widely used and cost-effective method, while PAG tests offer a blood-based alternative. The choice of method may depend on factors such as the stage of pregnancy, equipment availability, and the expertise of the personnel conducting the diagnosis. Combining multiple methods can sometimes enhance overall accuracy and reliability. The revived data showed that the RIA method were very precise for measuring bPAG concentrations in the maternal blood of the cows. The bPAG in blood as pregnancy test showed the possibility to replies the traditional rectal control or USG method. We confirm the good correlation between the results from the blood, rectal palpation and USG in pregnancy diagnosis. These three methods complementary each other.

1462 - Effect of parenteral micronutrient supplementation at birth on immunity, growth, and health in pre-weaning dairy heifers

Author: Angel Abuelo , Hannah Carlson, Faith Cullens-Nobis

Objectives
This randomized clinical trial aimed to determine the extent to which injectable micronutrient supplementation at birth can improve intranasal vaccine response by ameliorating oxidative stress in dairy calves from birth to weaning.
Material and methods
120 Holstein heifer calves were enrolled at birth and randomly allocated into one of four groups. The four groups included three commercially available micronutrient supplements (Selenium, Copper, Zinc, and Manganese; Selenium & Vitamin E; and Vitamins E, A, and D) and one control (saline). Calves received an intranasal vaccine against the respiratory viruses parainfluenza 3, bovine herpesvirus type 1 (BHV-1), and bovine respiratory syncytial virus (BRSV) within the first wk of life. Weight (BW) and hip height (HH) were recorded, and a blood sample and nasal secretion sample were collected at birth prior to treatment and vaccine administration as well as weekly until weaning at 8 wk. Health scores, including thoracic ultrasound assessment, were recorded weekly from wk 1 to wk 8. Farm treatment records were collected after the completion of the study. Serum micronutrient concentrations were determined from birth to weaning to identify micronutrient status, and serum blood metabolites were analyzed as markers of nutrient utilization. Redox balance was determined in serum as a ratio of reactive oxygen and nitrogen species (RONS) to antioxidant capacity (AOP), known as the oxidant status index (OSi). Intranasal vaccine response was quantified as anti-BRSV and anti-BHV-1 immunoglobulin A (IgA) concentrations in nasal secretions. Linear mixed models with repeated measures were built for the following outcome variables: micronutrient concentrations, blood metabolites, redox balance, IgA concentrations, BW, and HH. Pre-planned contrasts of control vs supplemented were also built for the primary outcome of IgA concentrations. A logistic regression mixed model was built for health events and treatment of disease.
Results
Serum selenium concentrations were greater in calves receiving supplements containing Se throughout the first 4 wk of life. However, we did not observe any consistent differences in the other micronutrients. The metabolic biomarkers indicate that supplemented calves had better energy status, as suggested by lower BHB and non-esterified fatty acids (NEFA) concentrations. Supplemented calves showed improved redox balance, as indicated by lower OSi throughout the first week of life. Calves supplemented with antioxidants at birth had higher anti-BRSV IgA than control calves. Our results indicate an improved immune response to vaccines in calves supplemented with antioxidants at birth. However, this did not translate to growth and health performance as there were no differences in average daily gain (ADG) or incidence of health events throughout the pre-weaning period.
Conclusions
This study provides evidence that improving the antioxidant capacity might improve vaccine response, and further research is required to investigate the appropriate frequency and dose of supplementation to improve calf growth and health.

1591 - Effect of a T-cell immunomodulator on the percentage of immune cells in the uterus of dairy cows during the puerperium

Author: Juan Manuel Guillen-Muñoz, Zurisaday Santos-Jimenez, Ramiro Gonzalez-Avalos, Juan Luis Morales-Cruz, Hugo Zuriel Guerrero-Gallego, Jesus Oliva-Guerrero

Objectives
The objective of this research was to evaluate the effect of a T-cell immunomodulator and its effect on the percentage of neutrophils, lymphocytes, and monocytes in the uterus of dairy cows during the puerperium.
Material and methods
The research was conducted from March to June 2023 at a dairy farm in Torreón, Coahuila, located in the semi-desert region of northern Mexico, at a latitude of 26°N. The management of the experimental units used in this study was in strict accordance with the guidelines for the ethical use, care and welfare of animals in research at the international (FASS, 2010) and national level (NAM, 2002). Eighteen multiparous Holstein cows from 3 to 6 parturitions and a body condition of 3.2±0.5 were used. The animals were randomly divided into two groups: the treated group (TG, n=9), which received 5 ml intramuscularly of a T-cell immunomodulator for veterinary use (I-MTC-VET®, Proquivet, Mexico); and the control group (CG, n=9), which received 5 ml of physiological saline solution at the time of delivery. Uterine sampling was performed using a sterile 5 mm endocervical brush (cytobrush). Once the sample was taken, it was immediately sent to the laboratory for evaluation. The variables evaluated were the percentage of leukocyte cells; segmented neutrophils (SEGs), lymphocytes (LI), monocytes (MO) and band neutrophils (BN) at 35 days postpartum. The comparison of variables between treatments was performed by means of an unpaired t-student test, using the R program version 4.0.5.
Results
The results obtained show that the group treated with immunomodulator did not show differences in SEGs with respect to the CG (39.22±3.6 *vs.* 40.22±5.7, respectively; P>0.05). Regarding LI, there were no differences between TG and CG (53.78±3.8 *vs.* 46.89±3.7, respectively; P>0.05). With respect to MO there was also no difference between TG and CG (6.89±1.9 *vs*. 5.0±1.1, respectively; P>0.05). Finally, in BN there was a significant difference between CG and TG (8.11±7.6 *vs.* 0±0, respectively; P<0.05).
Conclusions
In conclusion, the use of an immunomodulator at the time of delivery, decreased the presence of banded neutrophils at 35 days postpartum in the uterus of Holstein dairy cows. This type of cells is present when there is a severe infection. This could have important implications by decreasing the presence of severe uterine diseases during the postpartum period, helping to improve health during the postpartum period.
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1651 - Factors Influencing Early Growth and Health in Dairy Female Calves: A Comprehensive Analysis from Dairy Farms in Brazil

Author: Tadeu Eder da Silva, Joao Costa , Polyana Rotta, Carla Bittar, Sandra Gesteira, Rafael Azevedo

Objectives
The growth and health of dairy calves are important factors associated to the overall efficiency and welfare of dairy herds. This study aimed to investigate key variables influencing the weight gain of dairy calves and explore factors associated with the occurrence of prevalent diseases bouts such as diarrhea and respiratory diseases at 60 days of life.
Material and methods
A dataset composed of 10,395 records from 49 dairy farms in Brazil was used in this study (AltaCRIA program). To investigate the factors that affect calf body weight at 60 days of age, a full linear mixed regression model was built consisting of birth weight (kg), duration of gestation (days) and its squared value, lactation number and its squared value, birth season, breed (Holstein, 7/8 Holstein-Gyr, and 3/4 Holstein-Gyr), number of events of respiratory diseases and diarrhea up to 60 days as fixed effects and farm as a random effect. The selection of variables was carried out using the backward method, considering an alpha of 10%. Similarly, to investigate the variables associated with the risk of diarrhea and respiratory diseases, a complete logistic model composed of the same factors mentioned in the previous model were tested, converting the number of events into binary variables (0 = non-occurrence; 1 = at least one occurrence). Variable selection for the logistic models was carried out using 95% confidence intervals (CI). All the analysis ware performed using R Core Team software version 4.2.3, where the model fitted using the *lme4* package and its *lmer* (linear mixed model) and *glmer* (generalized logistic mixed model) functions.
Results
The average birth weight was 38 kg (±4.83 kg), the average weight at 60 days was 84.2 kg (±13.4 kg), the average gestation length was 274 days (±4.84 d), the number of average number of lactations was 2.04 (median = 2; min = 1; first quartile = 1, third quartile = 3, max = 15), the average number of diarrheas was 0.98 bouts (median = 1; min = 0; first quartile = 1, third quartile = 1, max = 4), and the average number of respiratory diseases was 0.48 bouts (median = 0; min = 0; first quartile = 0, third quartile = 1, max = 6). Body weight at 60 days was affected by birth weight (0.944 (95% CI [0.895, 0.992])), gestation length (linear term = 8.076 (95% CI [5.09, 11.06); quadratic term = -0.014 (95 % CI [-0.019, -0.009])), number of lactations (linear term = 1.548 (95% CI [1.112, 1.985])); quadratic term = -0.141 (95% CI [-0.208, -0.073])), number of diarrheas (-1.138 (95% CI [-1.50, -0.770])), and number of respiratory diseases (-2.195 (95% CI [-2.490, -1.89])). The marginal r-squared was 18.8% and the conditioned on the farm was 43.8%. Respiratory diseases risk was affected by gestation length (linear term = -0.320 (95% CI [-0.354, -0.286); quadratic term = 0.0005 (95 % CI [0.0004, 0.0006])), number of lactations (linear term = -0.176 (95% CI [-0.288, -0.064])); quadratic term = 0.021 (95% CI [0.003, 0.038])). The marginal r-squared for respiratory diseases risk was 5.0% and the conditioned on the farm was 32.9%. Diarrhea risk was affected by number of lactations of the dam (-0.176 (95% CI [-0.288, -0.064])) and birth season, where Winter was significantly lower than Fall ($P<0.05$), but both were similar ($P\geq0.05$) to the others by the Tukey test.
Conclusions
The marginal r-squared for diarrhea risk was 3.0% and the conditioned on the farm was 39.9%. Thus, this study provides valuable insights into the factors influencing the early growth and health of Brazilian dairy calves, showing the major impact of dairy farm management practices and minor animal characteristics.

1406 - Evaluation of factors associated with immunoglobulin, fat, protein and lactose concentrations in colostrum from dairy cows

Author: Katharina Lichtmannsperger

Objectives
The aims of this study were to describe factors that are associated with immunoglobulin, fat, protein and lactose concentrations in dairy cow colostrum. We hypothesized that herd-level and cow-level factors are significantly associated with colostral immunoglobulin, fat, protein and lactose concentrations from dairy cows.
Material and methods
In total, 72 dairy farms in the province of Salzburg, Austria participated in the study and 1,050 colostrum samples were collected between November 2020 and January 2022. The farmers filled out an online questionnaire to gather information on herd-level management practices and cow-level factors, e.g. number of lactations, time lag between parturition and colostrum harvest. Immunoglobulins were measured using a digital refractometer, fat, protein and lactose were analyzed according to the procedure described by Mößler et al. 2021.
Results
The results showed that the season of calving was a significant factor affecting colostrum quality whereof the best colostrum was found in autumn. Cows in their 6^{th} or $>6^{th}$ lactation showed significantly better quality than animals in their 2^{nd} lactation. Cows showing *ante partum* colostrum leakage showed a lower quality than cows with no leakage. Colostrum harvested >6 hours *post-partum* showed significantly lower quality than ≤6 hours.
Conclusions
The results confirm the current recommendations on colostrum management. Season affected colostrum quality; further studies are needed to find out the cause-effect relationship.

1503 - Critical control points for colostrum contamination and prevalence of Mycoplasma bovis in first milking colostrum from Scottish dairy herds.

Author: Alexandra Haggerty , Colin Mason, David Bell, Katie Denholm, Tracey Anderson, Emily Silva

Objectives

To identify the main source of contamination for first milking colostrum, to allow farmers to focus their attention on these 'critical control points' with implications for calf health and welfare. Additional objectives were to measure the prevalence of *Mycoplasma bovis* in colostrum and to identify specific risk factors for colostrum contamination through survey work with enrolled farmers.

Material and methods

Eleven farms were conveniently enrolled from the client lists of two commercial veterinary practices in the Dumfries and Galloway region of Scotland. Farmers were asked to complete a short questionnaire at enrolment with the lead researcher in person detailing their colostrum management protocols on farm..

From each farm, individual animal first milking colostrum samples (no pooled colostrum) were collected:

- directly from each enrolled freshly calved cow (T=Teat samples)
- from the bucket into which first milking colostrum is collected (B=Bucket samples)
- from the next storage vessel in which the colostrum is stored (if present) (B2, B3=subsequent bucket samples)
- from the calf feeder at point of feeding (F=feeder samples)

Sample collection was carried out by farm staff trained in the Standard Operating Procedure for collection of colostrum samples at the critical points. Samples were stored on farm at -20◦C and transported on ice to the laboratory where they were tested for total bacterial counts (TBC) and total coliform counts (TCC) using sheep blood agar and MaConkey agar plates respectively. A subset of 79 F samples were tested using Polymerase Chain Reaction testing for *Mycoplasma bovis*at an external laboratory (Scotland's Rural College). To detect an actual prevalence of 5% *Mycoplasma* species in colostrum with a desired precision of 0.05 and confidence of 95%, 75 colostrum samples were calculated to be required.

For each source type, descriptive statistics were calculated for TBC and TCC. TBC and TCC results were then dichotomized into either failing below or exceeding industry thresholds. The industry thresholds for TBC and TCC of 100,000 CFU/mL and 10,000 CFU/mL respectively were used. A one-way repeated ANOVA was constructed to determine the difference in TBC and TCC between the source type (teat, bucket, feeder). Risk factors for teat and feeder contamination and for the difference in TBC and TCC values between each source type were explored by constructing multilevel linear regression models using a backwards, stepwise elimination process with farm and source type as random effects.

Results

Preliminary results indicate that Scottish colostrum was showed variability in contamination at harvest, storage and feeding. Feeder samples tended to be most contaminated. Teat samples had a mean TBC of 47,323.68 CFU/mL (SD = 160898.20 CFU/mL) and mean TCC of 29,522.00 CFU/mL (SD = 226,029.90 CFU/mL). The first storage buckets had a mean TBC = 3635847 CFU/mL (SD = 16008325 CFU/mL) and a mean TCC of 521743 CFU/mL (SD = 1826190 CFU/mL). The feeder samples had a mean TBC of 14837540.70 CFU/mL (SD = 40115798.40 CFU/mL) and a mean TCC of 6705389 CFU/mL.1 (SD = 24583612 CFU/mL). There was a

significance difference in TBC and TCC between the source type (TBC: 3,168 F= 4.08 p<0.01, TCC: 3.168 F=3.78 p=0.01).

From the teat samples, 7.89 % (n=12/152) and 17.76 % (n=27/152) failed to meet industry thresholds for TBC and TCC respectively. Of the first storage bucket samples 50.34 % (n=75/149) and 54.36 % (n=81/149) failed to meet industry thresholds for TBC and TCC respectively. Finally, of the feeder samples 71.21% (n=94/132) failed to meet the industry TBC threshold and 76.52 % failed to meet TCC threshold (n=101/132).

Several risk factors were significantly associated with differences in TBC and TCC outcomes including size of colostrum storage container, number of buckets used to store colostrum and how the teats were cleaned at harvest ($p < 0.05$)

Seventy-eight (98.73 %) of the colostrum samples tested negative for *Mycoplasma bovis.* One sample (1.27 %) was inconclusive, suggesting potentially low levels of *Mycoplasma bovis*.

Conclusions

Preliminary results indicate that colostrum samples become more contaminated throughout the harvest, storage and feeding process. By focussing their attention to detail on basic hygiene practices, farmers could improve colostrum cleanliness and consequently calf health and immunity.

In line with international literature, *Mycoplasma bovis* prevalence was low in first milking colostrum.

1441 - Environmental sampling for paratuberculosis screening: preliminary results in cow-calf herds during the stabling season

Author: Gilles Foucras, Ludivine Gonzales, Celine Pouget

Objectives

Paratuberculosis is an endemic disease in many countries. Identification of infected animals and herds relies on serum and fecal testing which are labor-intensive and costly approaches. The possibility of sampling the environment to screen endemically infected herds has been investigated in dairy farms, but poor knowledge is available for the beef sector. The present study was implemented to evaluate the possibility of using such an approach in cow-calf herds during the winter stabling period.

Material and methods

To evaluate the possibility of environmental testing for paratuberculosis screening, cow-calf herds were selected in the database of a surveillance program for paratuberculosis based on serology testing. Results of the annual campaign for one of the last three years were available for 600 herds, with a minimal herd size of 50 adult cows. At least 80% of the cows above two years of age had been serologically tested. After stratification according to the herd seroprevalence, we categorized herds with not a single positive result, herds with a seroprevalence lower than 2%, and those having a prevalence over that threshold during the former follow-up period. Some herds vaccinated against paratuberculosis were also included. A protocol of environmental sampling was elaborated that is adapted to the housing conditions of cow-calf herds. When herd seroprevalence was above 5%, pens reserved for calves aged less than three months were also sampled using the same approach. SteriSox were threaded on single-use overboots for manure sampling. Walking along the feeding area and around the drinking points was performed with as many steps as possible to cover the whole lap. Socks were removed using single-use gloves and put into a sterile bag before being sent to the laboratory. Samples were stored at 4°C for up to 48 hours or frozen if extraction was delayed. DNA extraction was done using ID Gene Mag Paratuberculosis extraction kit, and quantitative PCR (qPCR) was performed with ID Gene Paratuberculosis duplex (both from Innovative Diagnostics SAS, Grabels, France). Statistical analysis was done with R software.

Results

In total, 135 herds were environmentally tested following the newly developed procedure. Amongst them, seven were vaccinated herds. The mean herd size was 94 adult cattle, with Limousine, Aubrac, and Blonde d'Aquitaine as the main breeds. Forty-four herds had at least one positive sample, giving an observed herd prevalence of 34.4%. Positive herds had a seroprevalence of 6.7%, 95%CI: [5.2, 8.1]. Serological prevalence was 2.1%, 95%CI: [1.6, 2.5] in negative herds. Ct values of the qPCR varied widely and were not correlated with the herd seroprevalence. In detail, all (19/19) seronegative herds gave negative results for environmental samples indicating a good specificity of the approach. Amongst herds with a seroprevalence lower than 2%, only one had a positive result. For herds with a seroprevalence between 2 and 5% and above 5%, 22/54 (40.7%) and 21/29 (72.4%) were identified by environmental sampling, respectively. Environmental sampling does not permit an estimation of the prevalence but enables the detection of shedding herds, in particular when the prevalence of seropositive cattle reaches a threshold of 5%. Out of 25 herds with a seroprevalence above 5%, 11 (44%) had a positive qPCR result on the calf pen sample, while qPCR was negative for two of them in the adult area. This suggests that additional calf pen sampling may be beneficial to the detection of paratuberculosis-infected herds. Four out of seven (57%) vaccinated herds had a positive environmental test indicating that vaccination does not prevent heavy shedders.

Conclusions

Environmental sampling is a promising procedure to evaluate the presence of shedders in beef cattle herds. A single test may not be enough to achieve a high sensitivity of detection as described in dairy herds. Repeated sampling during the winter season and targeting calf pens may help increase the screening sensitivity above the promising results obtained in this study.

1296 - Efficacy of a multivalent parenteral marker vaccine as a booster of a BRSV intranasal vaccine by means of immunological response.

Author: Marta Gibert Lleixa, Sara Baila Martínez, Mariona Tapiolas Verdera, Marc Vila Poch, Héctor Santo-Tomás, Carlos Montbrau, Ricard March Massós, Joaquim Mallorquí Bagué

Objectives

DIVENCE PENTA (HIPRA, Spain) is a multivalent viral vaccine developed to protect cattle from an early age against bovine respiratory syncytial virus (BRSV), bovine herpesvirus type 1 (BoHV-1), bovine viral diarrhoea virus type 1 and 2 (BVDV-1 and BVDV-2) and parainfluenza-3 virus (PI-3). On the other hand, NASYM (HIPRA, Spain) is a monovalent BRSV vaccine registered to be used intranasally (IN) from first days of life and can be heterologously boosted intramuscularly (IM) 8 weeks later.

The aim of this study was to demonstrate the efficacy of the heterologous prime-boost of BRSV in DIVENCE after an IN administration of NASYM by means of immunological response, as an alternative to IM vaccination of NASYM in young calves.

Material and methods

A total of 18 calves from 8 to 20 days of age from a commercial dairy farm were randomly distributed into the two study groups according to the BRSV antibody (IgG) levels to ensure balanced groups. At study day D0, animals from both groups were vaccinated IN with NASYM. Eight weeks later (D56), calves from the NASYM group were vaccinated IM with NASYM while calves from DIVENCE group were vaccinated IM with DIVENCE.

Blood samples (whole blood) and nasal secretions were collected to assess the immunological response after IN and IM vaccinations. Samples were collected at different timepoints from the beginning of the study until 10 days after IM vaccinations. Whole blood samples were collected to assess cellular response at study day D66 and D77. Nasal secretions were analyzed for BRSV IgA antibody detection by means of ELISA (INgezim BRSV Compac, Ingenasa) and a policlonal anti-Bovine IgA:HRP conjugated (Bio-Rad). Whole blood samples from the DIVENCE group were analyzed to measure interferon gamma production by T-helper lymphocytes using a commercial ELISA kit (Bovigam, Ingenasa).

The statistical analysis was done using R software v4.3.1. A p-value < 0.05 was considered as the limit of statistical significance. Comparison between groups was performed by the nonparametric Wilcoxon Mann-Whitney test.

Results

Before vaccination, both groups presented low levels of IgA antibodies. After IN vaccination with NASYM, a similar increase on the IgA levels was observed in both groups (D35). No statistically significant differences were observed between groups.

A similar antibody response between groups was also observed after IM vaccination, either with DIVENCE or NASYM (D56 and D66). No statistically significant differences were observed in any of the timepoints.

Results of interferon gamma response (IRPC values) in the DIVENCE group at D66 and D77 were 145.65 ± 211.958 and 125.89 ± 168.710, respectively. As shown, calves primed intranasally with NASYM developed cellular response after the heterologous IM vaccination with DIVENCE, both on study day 66 and day 77.

Conclusions

The results obtained in this study demonstrate that one intramuscular dose of DIVENCE boosts BRSV immunity primed by NASYM administered intranasally. The heterologous prime-booster for BRSV showed equivalent efficacy in IgA production for both protocols and DIVENCE further demonstrated cellular response to BRSV when applied 2 months after the IN use of NASYM.

1231 - Age distribution and severity of corkscrew claws in 10 Danish dairy herds

Author: Nynne Capion , Kurt Bach, Christina Christensen, Amalie Meinert Rinken, Kirstin Dahl Pedersen

Objectives

Corkscrew claw (CC) is a deformity of the claw where a rotation towards the interdigital cleft causes the axial wall to be non-weightbearing displacing the sole partly with the abaxial wall. In severe cases CC can cause severe lesions and lameness. CC can be seen on all legs and claws. The objectives of this study were to identify which age groups are affected by CC and score the severity of CC in commercial dairy herds.

Material and methods

Ten dairy herds with a known presence of CC were selected. In each herd a sample of ten animals from the age groups 0-6 months, 7-12 months, 13-18 months, 19-24 months, 25-36 months and >36 months were included. All four feet were inspected and photographed, and CSC was scored on site. CC was scored according to the width of the visible part of the axial wall, inspected directly in front of the foot. Normal= axial wall not visible; mild CC= up to 2 cm of axial wall visible, moderate CC= 2.1-3.5 cm of the axial wall visible, severe CC= >3.5 cm of the axial wall visible.

Results

The dataset included records on 2305 claws from 590 animals. All but two herds housed their young calves on deep straw, and only 18 calves 0-6 months are included in this study. In the 0-6 months old calves 11% had mild CC, the youngest calf with CC was 4 months old. Severe CC was seen in 14 months old heifers and up to 67 months old cows.

Normal shaped claws accounted for 59%, 33% was scored mild CC, 6.5% was scored moderate CC and 1.5 % had severe CC. CC was almost evenly distributed between left and right side. In the front legs 39% had normal claws, 50.5% had mild CC, 8.9% had moderate CC and 1.4% had severe CC. In the hind legs 79.4% had normal claw shape, 15.5% had mild CSC, 3.9% had moderate CC and 1.5% had severe CC.

Most of the CC was seen on the medial claws (79.4%) and CC affecting both medial and lateral claw was the next common observation (11.7%), and the lateral claw was more rarely affected (8.8%) .

Conclusions

In herds with a known presence of CC, the deformity can be seen in mild form in heifers as young as 4 months and severe CC can be seen in 14 months old heifers. CC was affecting the majority of dairy cows in these 10 herds. CC was most common on medial front claws but was recorded on all claws. The severe CC was mostly seen in older cows. The etiology of CC is still largely unknown. To prevent CC affecting even young animals and possible affecting their wellfare and production, there is a dire need to identify possible risk factors in genetics, housing and management practices.

1032 - Decrease in type G immunoglobulins in the colostrum of dairy cows and suckling cows during the first 5 days after calving

Author: Clara Bourel, Raphaëlle Deffrenne, Geert Vertenten

Objectives

Newborn calves acquire immunity from colostrum which is a natural source of immune factors and nutrients. Colostrum is defined as a secretion collected from the first milking, while milk from subsequent milkings is referred to as “transition milk”.

The IgGs in the colostrum and in the transition milk provide local protection in the neonatal gut. That is why some dairy farmers extend the distribution of colostrum during the calf’s first days of life. As beef cow colostrum is likely more concentrated than dairy cow colostrum, we would expect beef transition milk to contain more IgG (and nutrients) than dairy.

Material and methods

The study was carried out in 4 different breeds on 20 farms: 30 Limousin cows (beef), 32 Charolais cows (beef), 29 Holstein (dairy) and 19 Montbéliardes (dairy).

The colostrum was sampled from the cow just after calving and before the calf started to suckle. Then the transition milk was collected for 4 days: twice a day for the dairy cows (during the milking time), and once a day for the beef cows (in the morning). In the beef farms, the calves were allowed to suckle their mother ad libitum.

The colostrum and the milk were immediately tested by the farmer with a BRIX refractometer and frozen (-20°C).

The frozen colostrum and transition milk were shipped to the laboratory (LABOCEA, Brittany, France) and the concentration of IgG tested with a digital BRIX and RID (Radial Immunodiffusion).

Results

The time between calving and first milking varied greatly among the breeds. The time was on average, 27 minutes for Limousin, 38 minutes for Charolais, 5h11 for Holstein and 4h56 for Montbéliarde.

The volume of milk produced was only evaluated for the dairy cows. This volume gradually increased over time. On average the milking volume of Holstein cows increased with the number of milkings starting at 6.9L (2-15L) and reaching 14L (6-23L) after the 10th milking. In the same way, on average the milking volume of Montbéliarde cows increased with the number of milkings starting at 7L (2-15L) and reaching 12L (4.5-22L) after the 10th milking.

IgG in colostrum and transition milk tended to decrease over the time for all breeds and there was no difference in concentration between the primiparous and multiparous.

A decrease in IgG was observed for both beef and dairy, but differed on average level between beef and dairy.

On average the IgG concentration of Holstein cows decreased with the number of milkings starting at 79.4 g/L and reaching 2g/L after the 10th milking (corresponding on Day 5). In the Montbeliarde breed, the trends were the same. On average the IgG concentration of Montbéliarde cows decreased starting at 67.1 g/L and reaching 2.5 g/L after the 10th milking (Day 5).

In the case of beef breeds, IgG concentration at first milking was slightly higher than the dairy. On average the IgG concentration of Charolaise cows decreased with the number of days starting at 104 g/L and reaching 7.6 g/L on Day 5. In the Limousin breed, the trends were the same. On average the IgG concentration of Limousin cows decreased starting at 89.7 g/L and reaching 5.6 g/L on Day 5.

In dairy the IgG concentration of the 4th milking on the 2nd day was on average 7.2 g/L, almost equivalent to the average concentration on the 5th day in beef, 6,62 g/L.

Conclusions

This study demonstrates that after calving the IgG levels in colostrum and transition milk that provide local and passive protection decrease over time. The decrease should encourage farmers to extend the colostrum distribution over multiple days. For some cows, the volume of the first milking is insufficient to cover calf needs over several days. If needed, the IgG content in the second and third milking is still valuable to provide protection.

1271 - Thoracic Ultrasonography (TUS) to Evaluate Respiratory Lesions in Transported Calves: Impact of Time, Breed, Sex, and Transport Cohort

Author: Luca van Dijk, Susanne Siegmann, Niamh Field, Katie Sugrue, Cornelis van Reenen, Eddie Bokkers, Gearoid Sayers, Muireann Conneely

Objectives

Each year, large numbers of Irish-born calves are transported to The Netherlands for veal production. During these prolonged transit periods, calves face extended fasting periods and high risk of exposure to pathogens, all of which can contribute to the development of bovine respiratory disease (BRD). As diagnostic tool, thoracic ultrasonography (TUS) provides a non-invasive and accurate means of detecting BRD-associated lung lesions in calves. Tracking of lung pathologies in long-distance transported calves has not been undertaken previously. This study aimed to investigate the prevalence of respiratory (lung) abnormalities through TUS scoring before and after transport and to assess the influence of breed, sex, and transport cohort on TUS findings, and their relationship to visual health scores.

Material and methods

Irish-born calves were transported in two transport cohorts from their dairy farm of origin (DF) or a commercial livestock mart (MA) to an Irish assembly centre, and subsequently by road and ferry to a control post in France, and finally to a veal farm in The Netherlands. TUS scoring was conducted by a trained operator at DF/MA, and at three timepoints after transport (1-3 days, 7-10 days, and 18 days post-arrival at the veal farm). TUS scores were assigned for the left cranial, left caudal, right cranial, and right caudal lung lobes and an overall score was based on the total severity of the lesions; 0= no pathology in any quarter, 1= comet tail artefacts in one or more quarters, 2= abscess formation in one or two quarters, and 3= abscess formation in three or more quarters or consolidation in one or more quarters. Concurrently, health scores (eye discharge, nose discharge, ear position, coughing, rectal temperature, and a sum of these scores) were recorded at each TUS scan.

In total, 64 calves were included in the analysis (Breed: n = 15 Beef cross, n = 49 Friesian, Sex: n = 9 Female, n = 55 Male, Transport cohort: n = 35 Journey 1, n = 29 Journey 2, Age at arrival: range 16d to 42d, Weight on arrival: 40.7kg to 70.7kg). Logistic regression analyses were employed to investigate the impact of time-point, breed, sex, and transport cohort on ordinal TUS scores while accounting for the effects of weight and age at arrival. Spearman correlation statistics were calculated to explore associations between TUS scores and various health parameters.

Results

In total, 67% of TUS scores were abnormal (>0); 27% were mild (1); 30% were moderate (2) and 10% were severe (3). Moderate/severe TUS scores (score 2 and 3) tended to be observed more frequently at 7-10 days post-arrival compared to DF/MA ($P=0.09$), but not between other timepoints (DF/MA: 36%, day 1-3: 39%, day 7-10: 41%, and day 18: 45%). Friesian calves showed more moderate/severe TUS scores than Beef cross calves at 1-3 days post-arrival (60% vs. 25% of calves; $P<0.01$) and displayed a similar trend at 7-10 days post-arrival (45% vs. 26%; $P=0.08$). Moderate/severe TUS scores were observed more frequently in transport cohort 1 compared to cohort 2 at 1-3 days (60% vs. 22%; $P<0.01$) and 7-10 days post-arrival (45% vs. 14%; $P<0.01$). Additionally, female calves had more moderate/severe TUS scores than male calves at 1-3 days (95% vs. 60%; $P<0.01$) and 7-10 days (76% vs. 45%; $P=0.02$) post-arrival on the veal farm. There was no to poor correlation between TUS scores and all health scores ($r = -0.03$ to 0.16).

The minimal change in TUS scores between time-points may be due to batch antibiotic treatments given post arrival at the veal farm, which is designed to aid in the healing of lung

tissue and thus decreased the prevalence of TUS-identified pathologies in calves post arrival. Similarly, these treatments improve the health of calves and decrease the prevalence of visual negative health parameters, which possibly affected the correlations between TUS scores and visual health scores in this study.

Conclusions

In general, this study confirmed the high prevalence of lung abnormalities in young calves at all time-points, and that TUS scores can be affected by transport cohort, breed, and sex. However, additional research using larger numbers of calves is required to assess changes in lung abnormalities over time and correlations between TUS scores and visual health scores.

1035 - On-farm evaluation of the neonatal hygiene management protocol using ATP bioluminescence

Author: Pleun Penterman, S. Plekkenpol, M. Andringa, Henk Kuijk, Geert Vertenten, Bart Sustronck

Objectives

The first weeks of life are critical in calf rearing and are associated with the highest mortality due to enteric and respiratory disease. A proper hygiene management protocol can assist in the protection of newborn calves by reducing the load of pathogenic bacteria to which the calves are exposed. The Royal GD in the Netherlands proposes the “Hygiene Check Kalf” to obtain an idea about the bacterial load in the calf’s environment and on the utensils used for colostrum storage and administration. This “Hygiene Check” is based on routine bacteriologic cultures but requires samples to be send to the laboratory. The adenosine triphosphate (ATP) bioluminescence meter has been advocated as a simple and useful tool for the evaluation of the degree of microbiological contamination of surfaces and liquids. The objective of the present study was to examine whether an ATP bioluminescence meter can be used as an on-farm tool to assess the degree of microbiological contamination of the newborn calf’s environment.

Material and methods

On 10 dairy farms in the Netherlands a Royal GD “Hygiene Check Kalf” was performed to evaluate the cleaning and disinfection protocol used in the calf raising units. On the same occasion ATP bioluminescence measurements, expressed as the number of relative light units (RLU) were performed on samples of the calf pens, the colostrum administration material (bottles, nipples, oesophageal tube) and the milk buckets using UltraSnap ATP swabs and a SystemSURE Plus meter (Hygiena™, California, USA). On each occasion, the mean of three consecutive ATP bioluminescence measurements was calculated and used for analysis. Optimal cut-off points for the ATP bioluminescence method to classify the calf hutches, the colostrum administration material and the milk buckets as “clean” or “contaminated” were determined using the “Hygiene Check Kalf” method as reference. Statistical analysis was performed in R (R Core Team 2022).

Results

The median ATP bioluminescence of the calf hutches was 67 RLU (range 22-1880 RLU). The median ATP bioluminescence of the colostrum administration material and the milk buckets was 315 RLU (range 9-7450 RLU) and 1170 RLU (range 15-8290 RLU) respectively.

The optimal cut-point for the ATP bioluminescence method was ≤50 RLU (AUC 0.80) to classify calf pens as “clean”. The material used for colostrum administration can be classified as “clean” when the ATP bioluminescence was ≤10 (AUC 0.54). The optimal cut-point for the ATP bioluminescence method was ≤150 RLU (AUC 0.76) to classify the milk buckets as “clean”.

Conclusions

The results of this study indicate that ATP bioluminescence measurements can be used as an on-farm screening tool to evaluate the cleaning and disinfection protocol used in the calf raising units on Dutch dairy farms.

1419 - Cattle health programmes: what does success look like?

Author: Liz Cresswell

Objectives

To understand the key ingredients for success when implementing preventative cattle health programmes.

Material and methods

The study was undertaken as Nuffield Farming Scholarship, which incorporates two years of travel, interviews, farm visits and independent study. The author travelled to Europe, Southern Africa, New Zealand, Brazil and the USA to investigate the study topic. Interviews and meetings were conducted with over 100 farmers, veterinarians, industry stakeholders and politicians.

Results

The investigation identified that a wide variety of cattle health programmes to control infectious diseases exist across the world. In Europe these tended to be formally structured, incorporating diagnostic testing, ongoing surveillance and preventative measures. They were often supported by government and aided by national cattle recording systems including databases. In countries where national cattle health programmes do not exist, farmers needed to work with veterinarians to take responsibility for the health of their own herds, including heightened biosecurity and tightly controlled vaccination programmes. Regardless of whether national support exists or not, industry-based cattle health programmes have arisen due to the commercial benefits of demonstrating disease-free or controlled status.

Across the spectrum of herds and systems visited, one key element emerged – attention to the basics of animal health. Ensuring that nutrition, breeding, stress-reduction and preventative disease control were optimal was more fundamental to achieving successful animal health than the implementation of the health programmes.

Bureaucracy and excessive paperwork were identified as a barrier to participation in health programmes. The use of technology and automated animal monitoring solutions had the potential help to reduce duplication of information reporting, and it was seen as increasingly important to incorporate advancing agritechnological solutions into preventative health programmes.

There were cost-benefits associated with participating in cattle health programmes which need to be assessed for all stakeholders to encourage participation. Costs were not just financial – social, economic, temporal, geographical and biological factors could impact the success of these programmes in controlling cattle diseases. Programmes which incorporated an understanding of the motivators and barriers of farmers tended to have higher rates of success than those that did not. However, regulation was required to achieve complete disease control or eradication at a national level and it did not always guarantee total success.

Conclusions

Recommendations for successful preventative cattle health programmes were formulated based on the outcomes of the two-year scholarship:

- For governments and regulatory bodies – have a clear framework, which identifies key responsibilities, milestones and outcomes. Incorporate capacity for flexibility and allow for individual farm/regional variation.

- For industry (including veterinarians, consultants, food processors and agricultural organisations) – non-animal health factors (e.g. social, geographic, biological, economic) which can impact animal health should be considered; implement bespoke solutions based on the knowledge and understanding of individual and regional farming operations as these are more likely to achieve success than blanket implementation of rigid frameworks. Access to data and technology can streamline this process.

- For farmers – focus on achieving 'good basics' of nutrition, breeding, stress-reduction and preventative disease control in order to underpin good animal health and a robust business strategy.
- All – communication is required between all stakeholders, and this is best facilitated where partnerships exist.

Preventative animal health programmes are complex and multifactorial to implement. However, they play a key role in controlling disease, driving up cattle health and welfare standards and providing a commercial trade tool. There are some key ingredients which if followed, can underpin success not only for the cattle industry but for other livestock sectors too.

1249 - Calf health and disease in Danish dairy herds practicing cow-calf-contact

Author: Kirstin Dahl-Pedersen, Mette Bisgaard Petersen , Nynne Capion

Objectives
Dairy production systems with prolonged cow and calf contact (CCC) are slowly gaining more interest among farmers in part due to a growing consumer demand for more natural rearing. So far, a limited number of studies have investigated calf health and disease at farms with CCC. The objective of this study was to gain a better understanding of the potential effects of CCC on calf health.

Material and methods
In 10 Danish dairy farms practicing CCC the method of CCC was described during a visit to the farm. From the Danish national cattle database data related to disease and mortality during the first 120 days of the calves' lives were collected. All farms had started practicing CCC within the last 3-5 years and based on their own historical data we will compare the number of disease treatments and mortality rates before and after switching to CCC systems. In addition to health data, serum IgG was measured on in total 100 calves from four of the ten herds to evaluate passive transfer.

Results
Preliminary results show that calf serum IgG levels in the four CCC herds are above national average for herds not conducting CCC. Preliminary analysis of the effect of CCC compared to conventional rearing methods on calf health and mortality vary between farms. Some herds experience better health and lower mortality with CCC than before, and some herds experience the opposite. The calves in CCC systems seem to achieve sufficient passive transfer and the variability in calf health could be due to management factors. This particularly involves routines for early detection of disease in calves, which might be more challenging in CCC, where the daily milk feeding of the calves cannot be used to monitor general demeanor and health.

Conclusions
This study shows that calf health and mortality vary when herds switch from conventional rearing to CCC. Herds with improved calf health with CCC compared to conventional rearing can provide examples for others to follow. This study will provide a greater insight into the possible effects of CCC on calf health. This could form the basis for a better understanding of some of the potential barriers and challenges that farmers as well as advisers and veterinarians may experience when considering implementing CCC on dairy farms.

1234 - Identifying risk factors and factor blocks for calf mortality in large commercial dairy herds using multiblock analysis

Author: Dagni-Alice Viidu , Triin Rilanto, Tanel Kaart, Stephanie Bougeard, Kerli Mõtus

Objectives

Calf mortality is a common and increasing calf rearing issue in the dairy industry. Intensification of the dairy farms entails more challenging environmental and rearing conditions with concomitantly decreased individual attention allocated for each animal. Having progressively broader knowledge of the best calf rearing practices is needed to reduce production losses under these circumstances, to ensure good animal health and welfare, and profitability of the production.

This study aimed to provide an overview on calf mortality risk factors in a more comprehensive form by using a relatively new method in veterinary epidemiology called multiblock partial least squares (mbPLS) analysis. The mbPLS model simultaneously identifies the importance of individual variables as well as the composed thematic blocks and allows to use composite outcome including several variables that describe the same phenomenon, thereby increasing the number of dimensions of the gained information. This added layer of data will highlight the most important focus areas when tackling the complex problem of preweaning calf mortality.

Material and methods

The study included 120 Estonian dairy herds with at least 100 cows and a loose-housed keeping system for milking cows. Data was gathered between August 2019 and July 2020 by registering different herd management practices and performing on-farm measurements and animals' cleanliness scoring. Samples of bulk tank milk, heifers' and calves' blood, and calves' faeces were also collected to detect the endemic spread of the main pathogens in the herd and to determine the IgG1 level in calves' blood. Based on the farm records and Estonian Agricultural Registers and Information Board data, a yearly calf mortality risk was calculated for each participating herd for calves up to 21 days (MR21) and 22-90 days (MR90).

The 150 variables used in the analysis were aggregated into 13 thematic blocks and screened for univariable associations with MR21 and MR90 using negative binomial regression analysis adjusting for herd size. Variables with an unconditional p-value of < 0.2 were included in the mbPLS analysis where a composite outcome consisting of MR21 and MR90 was used. A total of 53 variables from 13 thematic blocks remained in the final mbPLS model.

Results

The mbPLS model explained 67% of the total variability in herd preweaned calf mortality. The most influential thematic blocks were "Calf housing conditions during the neonatal period (five days to three weeks of age)" (block importance index = 8.7%, 95% CI 5.6; 12.8), "Biosecurity practices" (8.4%, 95% CI 3.8; 12.7), "Routine stress-inducing activities" (11.6%, 95% CI 5.1; 20.1) and "Herd characteristics" (10.9%, 95% CI 6.1; 17.4).

The most influential variables in the mbPLS model associated with the composite outcome (mortality during the whole preweaning period) were having ≥10% of cows in the calving pen with dirty udder area and calves having access to outdoor area during the first 21 days of their life. These were both risk factors for MR90 while outdoor access was protective for MR21. Measuring the quality of the first colostrum, always washing the calf boxes between calves, and vaccinating cows against bovine herpesvirus 1 entailed lower MR21 and MR90.

The most prominent risk factors for solely MR21 were dehorning all calves (bulls and heifers) and dehorning at the age of 21-29 days whilst dehorning at ≥30 days of age resulted in lower MR21. Having the calf barn built or renovated within the last 10-20 years was also associated with lower MR21. Regarding MR90, the most important detected risk factors were higher daily quantities of milk offered to the calves after three weeks of age, using calving pen also for sick (or soon to be culled) animals, and feeding calves with waste milk (collected from cows receiving

antibiotic treatment or in a withdrawal period) during the first three weeks of calves' life. Higher average milk yield in the farm was associated with lower MR90.

Conclusions

Using multiblock model opened a new perspective in calf mortality risk factor analysis by providing novel information on the importance of different thematic blocks on calf mortality. The study results highlighted the importance of activities that improve calf resilience, reduce stress in the early neonatal period, and impede within-herd transmission of infections.

Funding

This work was supported by the Estonian Research Council grant (PSG268).

1747 - Effects of Solanum glaucophyllum and Calcium Salt Boluses Administered At Calving on Plasma Calcium Concentrations in Cows fed Diets With and Without Added Anions.

Author: Jesse Goff

Objectives

Hypocalcemia is a common disorder of transition dairy cows. Oral Ca boluses are currently available for use which contain soluble Ca salts and can raise plasma calcium (Ca) concentration using the paracellular intestinal Ca absorption pathway. But the effect only lasts for 4- 10 hr, depending on source of Ca and the amount administered. *Solanum glaucophyllum* (SG) is a plant containing a biologically inactive glycoside form of 1,25-dihydroxyvitamin D (1,25(OH)2D). In ruminants, the rumen bacteria cleave off the glucose residues, liberating the active form of vitamin D. As the 1,25(OH)2D is absorbed across the apical membrane of the intestinal tract it rapidly stimulates active transport of diet calcium (Ca) across the intestine, increasing plasma Ca concentrations from 12 to 96 hrs after administration. A bolus that combines a high dose of readily soluble Ca salts with the SG leaf was developed and tested on dairy farms.

Material and methods

The experimental boluses were tested on two large dairy farms. One farm (Holstein and Jersey) fed a prepartum diet containing anionic salts to reduce hypocalcemia after calving, the second Holstein farm did not feed anions. On both farms multiparous cows were randomly assigned to one of these treatments: Two experimental Ca + SG Boluses at calving only (74 g Ca total); commercial Ca Bolus (43 g Ca at each dose) administered at calving and again 12-24 hr later; and No Bolus treatment. Blood samples were collected from each cow at calving before bolus administration, and at 12, 24, 36, 48, and 72 hr after treatment. Plasma Ca concentration was determined by spectrophotometry using the Arsenazo III method. Subclinical hypocalcemia was defined as plasma Ca concentration below 8 mg/dl.

Results

Cows fed anionic diet before calving had higher plasma Ca concentration prior to treatment than cows fed No Anion diet (7.5 vs 6.4 mg/100 ml). Cows fed the anionic diet receiving the Ca+SG boluses at calving had significantly higher plasma Ca concentration than cows receiving No Bolus or the Ca Bolus at 24, 36, and 48 hrs after administration. Mean plasma Ca of anionic diet cows receiving the Ca+SG Boluses exceeded 8 mg/dl by 12 hr. Cows in the Ca Bolus and No Bolus treatments had mean plasma Ca concentrations below 8 mg/dl for the first 72 hr after calving. Cows fed the No Anion diet receiving the Ca+SG boluses had significantly higher blood Ca concentration at 12, 24, 36, and 48 hr after treatment than did cows receiving commercial Ca Bolus and No Bolus treatments. The No Anion diet, Ca+SG Bolus cows had mean plasma Ca that exceeded 8 mg/dl by 36 hr after treatment, while plasma Ca concentration of cows in the other two treatment groups did not reach 8 mg/dl during the first 72 hrs after calving. No cows receiving the Ca+SG boluses developed clinical hypocalcemia (milk fever) on either trial.

Conclusions

In this study, cows fed prepartum Low DCAD Anionic diets have improved plasma Ca concentration over cows fed High DCAD diets without anions at the time of calving, but they often still have a high incidence of cows with subclinical hypocalcemia (Ca below 8 mg/dl) at 2,3 and 4 days into lactation. The Ca+SG Bolus cows fed the High DCAD diet without anions had higher mean plasma Ca concentrations from 24 to 72 hr after calving than did cows fed the Low DCAD Anionic diet with or without commercial Ca boluses being administered . Our results suggest that the administration of 2 Ca+SG boluses to cows fed No Anion diets improves plasma Ca more reliably than does a Low DCAD anionic diet. The Ca+SG boluses are administered a single time to multiparous cows only, so the cows only need to be handled a single time to reduce hypocalcemia.

1396 - Comparative use of Automated Behavior Monitoring System (ABMS) versus on-Farm Standard Operation Procedure (SOP) for youngstock health in a commercial dairy farm

Author: Laura Elvira, Juan Pedro Campillo Beneitez, Carolina Tejero, Andre Preto, Eran Friedman, Mª Luz Sales

Objectives

Youngstock healthcare control is one of the biggest challenges for dairy farms. Many precision dairy technologies (PDT) have started to be used on dairy farms around the world initially dedicated for cows but now also for youngstock (Friedman et al., 2022). It is a useful tool for farmers and veterinarians to face the multiple new challenges of the sector, such as the lack of skilled labor, increase in rearing costs or new legislation focus on reduction of antimicrobial use.The aim of this trial was to compare the use of an automated behavior monitoring system (SenseHub™ Dairy, MSD Animal Health) versus an intensive on-farm standardized health scoring system (Control) on youngstock health and growth.

Material and methods

The study was performed on a large dairy farm (148 calving per month on average) located in Lleida (Spain). A total of 805 female calves born between February and November 2022 were enrolled at birth and followed up until seven months of life. Calves were randomly allocated to one study group: 1) automated behavior monitoring system (SHY) or 2) daily check using on-farm SOP (Control).

Preweaned calves were reared in individual hutches and fed twice a day, 6 liters/d of milk replacer with nipple bottles. After weaning calves were grouped in small pens (6 to12 calves until 98 days) and regroup later (30 calves until 112 days and 60 calves until the end of the study).

For calf health monitoring, in the control group, calves were diagnosed and treated according to on-farm SOP. Preweaned calves were checked by an on-farm veterinarian twice a day at feeding time using Wisconsin Calf Health protocol; and after weaning calves were daily checked visually and weekly by rectal temperature. Additionally, at weaning lung health was checked by ultrasound, and following Adams and Buczinski (2015) score, calves with a score higher than 3 were treated. On the other hand, in SHY group, an ear monitoring tag (SenseHubTM Monitoring Ear Tag, MSD Animal Health) was applied at birth to automatically monitor calf's behavior. The system calculates individual health index every hour, and twice a day (morning and afternoon) a list of calves with health index (HI) < 86 was printed and these calves were examined by a veterinarian following the same on-farm SOP.

In both groups, the following information was collected:

- Health status: morbidity, age of onset, treatment, and mortality
- Growth: body weight at weaning and 8 months of life.
- Labor time for checking and examining calves.

Results

Although in this farm, health is daily check by a youngstock on-farm veterinarian, the morbidity for the most prevalent diseases in this period (diarrhea and pneumonia) was similar in both groups. However, differences were observed in the days to diagnosis. The earlier first BRD diagnosis in preweaned calves in SHY group (56 vs 72 days old, respectively), with slightly higher percentage of calves detected and treated (12.3 vs 10.3, respectively). Moreover, lung ultrasound at weaning showed a lower percentage of mild and severe lesions in the SHY group (9.8 and 1.5 for SHY vs 14,2 and 5.8 for Control, respectively).

No significant differences among both groups were observed for ADG. However, the losses (mortality & culling) was doble in control group (2.63%) vs SHY group (1.20 %). The cause of this increment was the culling of heifers for poor growth (1.2 % at 195 days of life on average).

Conclusions

In conclusion, and from a practical point of view, the group monitored by the automated behavior monitoring system (SenseHub™ Dairy) delivered insights that helped the veterinarian to have an earlier BRD diagnosis and lower prevalence of lung lesions at weaning, with a positive impact in a better use of antibiotics and long-term impact of the disease, as we could see with the higher culling rate in the control group. Moreover, in the control group, more time and experience were needed to identify sick calves; while SHY reduced the labor time spent to check calves, as the work was only focus on those calves previously identify by the alerts.

1702 - Productive characterization and somatic cell count of Holstein cows under intensive grazing in the Querétaro highlands in Mexico

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Objectives
Based on some productive variables and the somatic cell count, carry out the characterization of Holstein cows under intensive grazing conditions.
Material and methods
The variables of number of lactations, duration of lactation (days), onset of pregnancy (days after lactation began), dry period (days), interval between births (days), moment of lactation (day), milk production (kg) and Somatic Cell Count of 612 samples, taken over four years (2008-2012) from 49 Holstein cows under intensive grazing conditions belonging to the Centro de Enseñanza, Investigación y Extensión en Producción Animal en Altiplano de la Facultad de Medicina Veterinaria y Zootecnia from Universidad Nacional Autónoma de México located in Tequisquiapan, Querétaro. The characterization was carried out using a principal component analysis (PCA) for variable reduction and cluster analysis (CA), based on the nearest neighbor, for the generation of groupings. The retention criterion for the principal components was an eigenvalue greater than 1.00 and a Euclidean distance of 1.01 for the formation of the groupings.
Results
The average variables are the following: The lactation number was 2.00, with a gamma distribution, the duration of lactation was 346.20 days, the start of pregnancy once lactation had started was 134.30 days, the dry period was of 62.62 days, the interval between calving was 408.80 days, the days in milk were 175.10, the milk production was 20.00 and the somatic cell count was 120087. Four principal components were retained that cumulatively explain 89.65% of the data variability. Component 1 is composed of the variables duration of lactation, time of onset of pregnancy and interval between births; Component 2 contains the variables day of lactation and milk production; Component 3, of the variables number of lactation and duration of the dry period and Component 4 only considers the somatic cell count. Four groupings resulted from the CA; Group 1 included 309 samples and had the following averages: the number of lactations was 1.82, the duration of lactation was 322.07 days, the start of pregnancy once lactation had started was 108.70 days, the dry period was 61.12 days, the interval between calving was 383.20 days, the days in milk were 188.69, the milk production was 20.90 and the somatic cell count was 350497.43, this value indicates that the udder health of this group is mastitis subclinical; Group 2, with 222 samples, had averages of: 1.70 lactations, the duration of lactation was 334.06 days, the beginning of pregnancy once lactation had begun was 119.93 days, the dry period was 60.36 days, the interval between births was 394.43 days, days in milk were 175.95, milk production was 18.96 and somatic cell count was 88592.23; Group 3, with 39 samples, had on average: the number of lactation 2.45, the duration of lactation was 374.02 days, the start of pregnancy once lactation had started was 160.87 days, the dry period was 61.35 days , the calving interval was 435.37 days, the days in milk were 174.83, the milk production was 21.14 and the somatic cell count was 119220.72; and Group 4, with 42 samples, on average showed a lactation number of 2.04, the duration of lactation was 310.16 days, the start of pregnancy once lactation had started was 123.09 days, the dry period was 87.42 days. , the calving interval was 397.59 days, the days in milk were 157, the milk production was 20.78 and the somatic cell count was 142428.57.
Conclusions
The multivariate statistical methods of principal component analysis and cluster analysis allowed us to efficiently characterize the production variables, which initially seemed to have no patterns. Principal Component 4, which only considered the somatic cell count in its structure, allowed us to identify the udder health of the groups.

1735 - Tools to estimate body weight and growth in dairy heifers

Author: Emelie Ahlberg, David Alejandro Contreras Caro del Castillo, Dorota Anglart, Carlos Hernandez, Sigrid Agenäs

Objectives

The aim of this study was to evaluate different tools to estimate body weight and growth in dairy heifers.

Material and methods

Data were collected at 6 different occasions between October 2022 and January 2023 and was performed at the Swedish Livestock Research Centre in Uppsala, Sweden. Data collection included a total of 165 dairy heifers of two breeds: 96 Swedish Red and 69 Swedish Holstein in the age range between 6 and 15 months. Four different measuring tools were used for the body measurements: a measuring tape (cm), a weight-by-breed dairy cow tape (cm and kg) (The Coburn Company, Inc., Whitewater, WI), a hipometer caliper (kg) (Dairy Innovations, Alexander, NY) and a sliding-scale height stick (cm) with a spirit level. Body weight (BW) and nine different body measurements, body length, chest girth (CG), hip width, backside width, ischial width, hip ischial width, withers height, hip height and external width between the hip joints, were collected. Heifers with a full set of manual body measurements and BWs from the scale (n=46) were used in the statistical analysis.

Results

Pearson correlations were used to investigate the relationship between each body measurement and BW. The highest correlation was found between BW and CG (r = 0.94), and BW and hipometer (r = 0.91). Hip ischial pin (r = 0.82) and hip height (r = 0.79) were also found among the highest correlations with BW. The growth rate was calculated as % increase in BW of each heifer between the data collections. Both the highest and the lowest growth rate was observed in the SRB. The maximal growth rate was found to be 16% at the age of 7 months while the lowest growth rate was found to be -10% at the age of 8 months. The manual collection of body measurements was important for understanding which body measurement correlated to BW and what tool had highest accuracy estimating BW in dairy heifers. Our findings were in accordance with previous studies estimating BW using CG as a proxy where CG has shown to be more accurate than other measurements (Davis *et al.,* 1961; Heinrichs *et al.,* 1992) with correlations from 0.82 to 0.92 (Yan *et al*., 2009; Gruber *et al*., 2018; Tebug *et al*., 2018).

Conclusions

We conclude that manual collection of several body measurements can be valuable to estimate the BW of dairy heifers, however these methods are largely time consuming. Automatic tools for monitoring growth of dairy heifers are crucial for follow up and precision management, and this work provides insight into which body measurements have the potential to automatize as growth monitoring tools of dairy heifers.

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1428 - Analysis of In-Line Registered Milk Fat-to-Protein Ratios as Biomarkers for Assessing Metabolic Status in Dairy Cows

Author: Ramūnas Antanaitis , Karina Džermeikaitė, Vytautas Januškevičius, Justina Krištolaitytė

Objectives
Milk is highly promising as a diagnostic tool due to its non-invasive and routine collection during milking, supported by long-established standard analysis methods. Consequently, further investigation into early milk sampling and potential integration of new data sources is warranted to enhance predictive capabilities for early warning systems. Additionally, gathering and analyzing more data from a variety of farms is crucial to increase the robustness of the model against differences in management and feeding strategies . The purpose of this study is to detect variations in the milk fat-to-protein ratio recorded in-line, which is crucial for evaluating the metabolic health of dairy cows.
Material and methods
Throughout the duration of the study, farm visits were conducted every two weeks on set days. During these visits, the composition of the milk, particularly its fat and protein content, was analyzed using a BROLIS HerdLine in-line milk analyzer (Brolis Sensor Technology, Vilnius, Lithuania).
During each farm visit, coinciding with the clinical examinations, blood samples were collected in tubes without anticoagulants to evaluate GGT and AST activities and albumin levels. NEFA concentrations were determined using a wet chemistry analyzer. For measuring plasma levels of BHBA and glucose, blood samples from the ear were analyzed using the MediSense and FreeStyle Optium H systems. BHBA concentrations were monitored daily through blood sampling. These samples were all taken during the clinical evaluations. The cows in the study were divided into three groups based on their conditions: subclinical ketosis (SCK; n = 62), identified by increased milk fat-to-protein (F/P) ratios (>1.5 mmol/L) but no visible signs of other post-calving diseases; subclinical acidosis (SCA; n = 14), marked by low F/P ratios (< 1.2), severe diarrhea, and undigested feed in feces, absent other post-calving issues; and a healthy group (H; n = 20), consisting of cows showing no clinical symptoms and maintaining a normal milk F/P ratio of around 1.2.
Results
In the study, cows with subclinical ketosis (SCK) showed significantly elevated milk fat-to-protein ratios, averaging 1.66 (± 0.29; $p < 0.01$), which was notably higher than those in subclinical acidosis (SCA) cows (0.93 ± 0.1; $p < 0.01$) and healthy cows (1.22). This represented a 36% increase in the milk fat-to-protein ratio for SCK cows and a 23.77% decrease for SCA cows. Additionally, there were marked differences in AST activity, with SCA cows showing a 26.66% increase ($p < 0.05$) compared to their healthy counterparts. SCK cows also demonstrated a 40.38% increase in NEFA concentration ($p < 0.001$). A positive correlation was observed between blood BHBA and NEFA levels ($r = 0.321$, $p < 0.01$), alongside a negative correlation between BHBA and glucose concentrations ($r = -0.330$, $p < 0.01$). Furthermore, a strong positive correlation was found between AST and GGT levels ($r = 0.623$, $p < 0.01$).
Conclusions
The findings indicate that alterations in the in-line milk fat-to-protein (F/P) ratio serve as reliable indicators for determining the metabolic status of dairy cows. This in-line F/P ratio is particularly effective in identifying cows at increased risk of Negative Energy Balance (NEB), as it shows a strong positive correlation with blood NEFA concentrations. Moreover, the in-line F/P ratio is useful in detecting cows at a higher risk of subclinical ketosis (SCK) and subclinical acidosis (SCA). Specifically, cows at a higher risk of SCK exhibited a 36% increase in their milk F/P ratio, while those at a higher risk of SCA showed a 23.77% decrease compared to cows not at risk of these conditions. These results demonstrate that comparing blood and milk metabolic data can

effectively use the milk fat-to-protein ratio to evaluate a cow's metabolic health. Milk characteristics are crucial in predicting metabolic stress in cows, as they align with blood markers of lipolysis and ketogenesis. Given that milk samples are collected non-invasively, they are particularly suited for routine assessment of metabolic status.

We believe this approach could significantly enhance dairy farm herd health programs. Monitoring the energy status of individual cows allows farmers to identify those at risk of metabolic stress, offering a valuable tool for proactive herd management.

Nutrition

1759 - Smectite supplementation to preweaned dairy calves: Effects on passive transfer immunity, morbidity, performance and mortality

Author: Ainhoa Valldecabres Inchaustegui, Noelia Silva-del-Río, Alexandra Correa, Marcelo B. Abreu

Objectives
Morbidity and mortality of preweaned calves is both costly and a health issue under the antimicrobial resistance global thread. Clays are naturally occurring, fine-grained geologic materials that can act as adsorbents, antioxidants, and pH buffers. The objective of this study was to evaluate the effects of supplementing preweaned dairy calves with smectite (montmorillonite bentonite clay) on serum IgG, morbidity and mortality.
Material and methods
Two-hundred Holstein and Jersey calves were assigned to control (CON; force-fed 50 mL of water using a syringe; n = 100) or smectite supplementation [SMECT; force-fed a smectite paste (2.5 g of smectite plus 5 mL of water) followed by 50 mL of water using a syringe; n = 100]. Researchers administered treatments 2 h prior to the afternoon milk feeding from 1 to 10 d of age. Jugular blood samples were collected at d 1, 4, 8, 12, and 16 of age for serum total protein, serum IgG and blood hematocrit determinations. Serum mineral concentrations (Ca, P, Mg, Cu, Fe, Zn) were determined at d 1 and 8 of age. Researchers assessed all calves' health (fecal consistency, appetite and respiratory disease) daily up to 20 d of age. Calves' therapeutic treatment by farm personnel and mortality records were collected up to 30 d of age. And body measurements (heart girth, body length, height at withers and height at hip) were taken at d 1, 12 and 60 of age. Continuous outcomes were analyzed by multiple linear regression and categorical outcomes were analyzed using logistic regression.
Results
A trend for a treatment effect conditional to time was observed for serum IgG concentration; serum IgG concentrations were higher for SMECT compared to CON calves during the supplementation period (22.4 vs. 18.9 g/L). Serum TP (5.3 vs. 5.2 g/dL) and mineral concentrations (in mmol/L; Ca: 2.7 vs. 2.7, P: 2.8 vs. 2.8, and Mg: 0.9 vs. 0.8; in µmol/L; Cu: 12.0 vs. 12.5, Fe: 31.5 vs. 41.0, Zn: 13.09 vs. 12.72) and blood hematocrit (31.3 vs. 31.1%) were similar among SMECT and CON calves. No statistically significant differences were observed on fecal consistency, appetite, respiratory disease, body measurements, therapeutic treatments or mortality. However, numerical differences in mortality (3 vs. 8%; Risk Ratio = 0.4 (95% CI = 0.1 to 1.3)) suggest that further research to evaluate smectite supplementation's potential to improve calves health is warranted.
Conclusions
In conclusion, smectite supplementation of preweaned dairy calves during the first 10 d of age did not result in negative health or growth implications, further, it may help to maintain serum IgG concentration while supplemented.

1252 - Evaluation of bovine colostrum replacer supplementation to improve weaning transition in Holstein dairy calves

Author: Kristen Edwards , Sylvia Bell, Stephen LeBlanc, Trevor DeVries, Michael Steele, João Costa, David Renaud

Objectives
Weaning of dairy calves from a liquid to solid diet often results in hunger, gastrointestinal tract challenges, and consequently a reduction in average daily gain during the process. Reduced growth negatively affects future production, thus creating a need for interventions to mitigate the negative effects of weaning. The objective of this randomized controlled trial was to evaluate the efficacy of supplementing bovine colostrum replacer during weaning as a method to reduce intestinal permeability and improve growth.
Material and methods
For this experiment, 65 calves were enrolled and housed individually until 70 d of age. Calves were fed milk replacer (150 g/L) three times daily with 9 L/d, 10.5 L/d, 11.25 L/d, and 12 L/d offered from d 1-7, d 8-14, d 15-21, d 22-56, respectively. Calves were weaned over 8 d from d 57 to 64, receiving a total of 7.8 L in two meals from d 57-60 and one feeding of 3.8 L from d 61-64. At d 57, calves were blocked by birth weight and randomly assigned to one of two treatments, equal in metabolizable energy, which were fed once daily during weaning from d 57-64: control (**CON**; n = 31): 3.8 L milk replacer (150 g/L) fed by nipple bottle, or colostrum supplementation (**COL**; n = 34): a mixture of 1 L bovine colostrum replacer (125 g/L) and 3 L milk replacer (150 g/L) with 3.8 L of the mixture fed by nipple bottle. Serum IgG was measured within 48 h of birth and bodyweight was taken at d 0, 57, 60, 64, 70, 77, and 84. Starter intake and respiratory score were measured daily from d 50 to 70 and fecal consistency was examined daily from d 56 to 70. Serum BHB and lung consolidation were evaluated at d 57, 64, and 70 and intestinal permeability was assessed by recovery of chromium-EDTA, lactulose, and d-mannitol from plasma after oral administration at d 56 and 65.
Results
There was no difference in body weight between treatment groups at the start of weaning (P = 0.97); however, COL calves were 2.79 kg (95% CI: 0.90 to 4.68; P = 0.004) and 2.76 kg (95% CI: 0.86 to 4.65; P = 0.004) heavier than CON calves at d 77 and 84, respectively. Additionally, COL calves tended to have improved ADG from d 57 to 84, gaining 100 g/d more than CON calves (95% CI: -10.41 to 207.13; P = 0.08). There were no differences in any of the other variables measured.
Conclusions
Calves that were supplemented with 125 g of bovine colostrum replacer once daily during the 8-d weaning period had improved growth two and three weeks after weaning. The results suggest that supplementation of bovine colostrum replacer during weaning may support growth, but the mechanism of action is not clear.

1164 - Description of different feeding strategies of transitional milk on five Danish dairy cattle farms

Author: Kenneth Krogh, Pedro Rodriguez Fernandez, Katja Hornbaek Mikkelsen, Lene Jensen, Hanne Skovsgaard Pedersen , Katrine Fynbo, Geert Vertenten

Objectives
Transitional milk feeding refers to the practice of feeding the milk obtained from 2 to 6 milkings after calving. This feeding practice can be beneficial for the gut health and local immunity of young calves. It also optimizes the use of dam vaccination against calf enteric diseases. However, systematic transitional milk feeding is not commonly practiced on modern dairy farms. The objective of this study was to assess transitional milk feeding practices of five dairy farms in Denmark. Additionally, the abstract proposes herd-specific checkpoints for assessing the effectiveness of transitional milk feeding.
Material and methods
Five Danish dairy farms, with a total of 2200 milking cows, were chosen for this study. The farms were selected based on their calf caretakers' motivation to improve calf health and their willingness to adopt new feeding strategies. The implementation of transitional milk feeding at the farms, involved three main aspects: Collection of milk (between 2 to 6 milkings post calving), storage (either through cooling, freezing, or immediate feeding without storage), and feeding strategy (one or twice a day, from 100mL to 4L and for 3 to 60 days). Transitional milk samples were collected at various stages of storage. The samples were tested for bacterial count by the herd veterinarian using either culturing or polymerase chain reaction (PCR) techniques. Additionally, the farms recorded data on colostrum feeding procedures for each individual calf, including the measurement of calf immunity through serum IgG levels measured by BRIX from day 1 to day 7. Moreover, a diarrhea score ranging from 1 to 3 (1: normal, 2: loose, 3: watery) was assigned to each calf based on the presence and severity of diarrhea.
Results
All five farms pooled their transitional milk, but each farm had different approaches to storage, feeding procedure, and feeding strategy. Regarding storage, Farms 1, 2, and 3 opted for cooling the transitional milk, while Farm 1 also utilized freezing. Farms 4 and 5 chose not to store the milk and fed it fresh. In terms of feeding procedure, Farms 1 and 4 fed the transitional milk separately, while Farms 2, 3, and 5, mixed it with whole milk or milk replacer. Each farm had a unique approach to transitional milk feeding to young calves as follows: 1L once a day for 3 days for Farm1; 200-500ml twice a day for 14 days for Farms 2 and 3; 4L twice a day for 5 days for Farm 4 and 100-500ml twice a day for 60 days for Farm 5.
Initially, three of the farms froze the transitional milk, but two of them transitioned to the practice of cooling the milk until feeding, with cooling times ranging from 12 to 72 hours. Bacterial count analysis of the transitional milk revealed bacterial growth during cooling on all farms. This finding suggests that all herds should prioritize swift and safe handling of the milk from milking to feeding to minimize bacterial contamination.
Serum BRIX, a measure of calf immunity, was assessed on all farms. Although the results varied between farms, there was no evidence to suggest that transitional milk feeding influenced serum BRIX levels.
To evaluate the impact of the new transitional milk approach, three farms conducted two rounds of diarrhea scoring. The results demonstrated a reduction in the occurrence of mild-severe (score 2-3) diarrhea following the implementation of transitional milk with a 46%, 55% and 100% reduction for Farm 2, 3 and 4 respectively.
Conclusions
The study concluded that handling and feeding of transitional milk can be tailored to individual farm conditions with a positive impact on diarrhea scores. Bacterial growth during cooling,

emphasizes the need for routine monitoring of milk quality when storing milk in the fridge. Despite bacterial growth, farms preferred cooling over freezing for time-saving reasons. More studies need to confirm, whether this approach is recommendable regarding disease transmission and preservation of bio actives in transitional milk.
When adopting new feeding approaches for young calves, monitoring colostrum feeding and passive transfer is crucial to ensure an optimal calf health and growth. Additionally, monitoring bacterial levels in transitional milk and performing diarrhea scores are important checkpoints for evaluating herd-specific transitional milk feeding strategies.

1247 - Nutritional emulsifiers as a potential digestibility enhancer in the ruminant metabolism

Author: Aurèlie Montagnon, Meissa Topete García Ulloa, Veerle Vandendriessche, Jolien van Soest, Alfredo Escribano

Objectives

The aim of the trial was to simulate *in vitro* the rumen conditions and to assess the impact of a nutritional emulsifier on the volatile fatty acids (VFA). It was hypothesized that the emulsifier wouldn't change VFA production.

Material and methods

A dairy cow ration, consisting of 30% grass silage, 30% maize silage and 40% concentrate, was dosed in triplicate, in sealed incubation flasks. A hydrophilic nutritional emulsifier [Excential Energy Plus, Orffa Additives B.V.], composed of glyceryl polyethylene glycol ricinolate (GPGR), was added to the incubation flasks, at four different concentrations (0, 25, 250 and 2500 µg/mL). Doses of 25 and 250 µg/mL were dissolved in dimethyl sulfoxide (DMSO). To correct for potential effect of DMSO on the rumen fermentation, it was added on-top to the flasks with 0 and 2500 µg/mL dosages. A CO_2-saturated bicarbonate/phosphate buffer with 5 mL of rumen fluid was added to the flasks to reach a total volume of 25 mL. The rumen fluid was sampled from three adult rumen fistulated sheep on a hay-concentrate diet, before morning feeding. Flasks were incubated at 39°C, to simulate rumen conditions, in a shaking incubator for 24 hours. At the end of incubation period, 1 mL of medium was collected and centrifuged for 15 min at 4°C, after which the supernatant was filtered. Finally, VFA were analyzed through gas chromatography (HP7890A), with a Nukol column and flame ionization detector. The production of VFA was calculated by comparing their concentrations in the rumen fluid before and after incubation. Linear and quadratic effects were tested.

Results

The increasing dosages of the nutritional emulsifier didn't show any effect on the total VFA production (P = 0.98, average = 1552 µmol/flask). The pH was also not significantly impacted (P = 0.92, average = 6.30). Related to butyrate production, a significant decrease was observed at the dose of 2500 µg/mL compared to the other treatments (P = 0.02). When looking at the molar proportions of the individual VFA, the higher dose of the nutritional emulsifiers also showed significantly lower acetate and butyrate (P = 0.04 and 0.003, respectively), and significantly higher propionate and valerate molar proportions (P = 0.01 and 0.05, respectively).

The nutritional emulsifier didn't show any impact on the VFA production and pH, and the effects on VFA molar proportions were only observed at the highest dosage. It seems that this feed additive will not impact the ruminal fermentation and microbiota when fed to ruminants, when fed at recommended dosage (corresponding to 250 µg/mL).

Conclusions

The effects of lipophilic emulsifiers, such as lecithins, have already been proven in dairy and beef cattle (De Nardi et al., 2012; Wojtas et al., 2020). However, the research on nutritional emulsifiers based on GPGR are still limited in ruminants. There is a potential for this type of emulsifier to be active in the small intestine to enhance the absorption of lipids, benefiting the productivity of cattle. This *in vitro* study shows the neutral effect of the nutritional emulsifier in the rumen.

1198 - Megasphaera elsdenii NCIMB 41125 capsule administration effects on growth performance in Holstein calves: preliminary results

Author: Nathaly Carpinelli, Marcello Guadagnini, Osvaldo Sousa, Valdir Chiogna Junior

Objectives

Maximizing growth during the preweaning period in dairy calves has shown to influence productivity during the first lactation. Weaning implies the full transition from liquid feed to solid feed, and this can be a stressful process. *Megasphaera elsdenii* is an anaerobic bacterium largely present in the ruminal population of healthy cattle. NCIMB 41125 strain has been selected for the ability to metabolize lactic acid and produce butyric acid, which promotes greater reticulorumen development and papillae growth. The present study aims to address the association between *Megasphaera elsdenii* NCIMB 41125 capsule administration and growth performance during the preweaning phase in Holstein calves.

Material and methods

On a single high producing dairy farm in Goiás state (Brazil), 96 Holstein female calves born from January to May 2023 were randomly assigned to either a treatment (LACT; n=49) and control group (CON; n=47). Calves belonging to LACT group were administered a single oral capsule containing 5 x 10^9 CFU of freeze dried *Megasphaera elsdenii* NCIMB 41125 [Lactipro FLX, Axiota Animal Health] at 55±6 days of age; CON calves remained untreated. During the preweaning period, calves were housed in individual hutches and fed 8 liters per day (2 meals/day) of whole milk enriched with milk replacer to achieve 20% dry matter (DM) for the first 30 days of life. Afterwards enriched milk was gradually decreased in two steps to 6 and 4 liters/day until complete weaning. Commercial starter feed (23% protein) and grass hay (5% of total DM) were offered *ad libitum* for the entire preweaning period. Weaning occurred at 89±3 days of age. Individual bodyweight (BW) was collected at birth, at 30 days of life and at weaning with a weigh tape. Average daily gain (ADG) was calculated between birth and weaning and between 30 days of life and weaning. Contingency analyses were performed to address differences among treatment groups for bodyweight at all time points prior to treatment. Two linear regression models were calculated with ADG from 30 days to weaning and from birth to weaning as independent variables. Dependent variables for the first model were BW at 30 days, treatment, weaning age and the interactions between treatment and BW at 30 days and treatment and weaning age. In the second model the dependent variables were: birth weight, treatment, weaning age and the interactions between treatment and birth weight and treatment and weaning age. Moreover, a final linear regression model was made with weaning weight as the independent variable and treatment, birth weight, weaning age and the interactions between treatment and birth weight and treatment and weaning age, as dependent variables. The statistical analysis was performed with JMP 16® (SAS), significant differences were declared at P <0.05 and tendencies at P <0.10.

Results

Birth weight was significantly higher in the CON group (41.4 Kg) compared to LACT (39.9 Kg) (P=0.02), but weight at day 30 did not differ (P=0.16). Age at weaning was greater for CON (90.0 days) compared to LACT (88.7 days) (P=0.04). Both models with ADG as independent variable were significant (P= 0.001 and P=0.002, respectively). Treatment was significantly associated with ADG from 30 days to weaning: ADG least squares means (LSM) were 1.204 kg/day (95% CI 1.162-1.246) for the LACT group and 1.089 kg/day (95% CI 1.045-1.134) for CON (P=0.0003). None of the tested interactions was significant. Similarly, treatment group was significantly associated with ADG from birth to weaning: ADG LSM were 1.086 kg/day (95% CI 1.052-1.120) for the LACT group and 0.879 kg/day (95% CI 0.842-0.915) for CON (P=0.00001). No significant interactions were calculated. Finally, the weaning weight model yielded significantly higher LSM

for LACT (137.7 kg; 95% CI 135.0-140.3) compared to CON group LSM (129.6 Kg; 95% CI 126.7-132.5) (*P*=0.00009).

Conclusions

This study demonstrated that the administration of a capsule containing *Megasphaera elsdenii* NCIMB 41125 during the preweaning period is associated with higher growth during the preweaning period and higher weaning weight in Holstein female calves.

1109 - Effect of feeding 5 days of transition milk compared with milk replacer on health status, growth rates of dairy calves.

Author: Katharine S Denholm, Geert Vertenten, Katharine Baxter-Smith, Michael Denholm, Paul Williams

Objectives

To compare average daily gains and health score outcomes between dairy calves fed an initial feed of 4-5 litres of first milking colostrum followed by 5 days of transition milk feeding (milkings 2-3) compared with calves fed an initial feed of 4-5 litres of first milking colostrum followed by milk replacer.

Material and methods

One hundred and fifty female replacement calves were enrolled on two Scottish dairy farms between May 2023 and November 2023. All calves were weighed at birth and received 4-5 litres of first milking colostrum within 4-6 hours of birth and were subsequently randomly allocated to one of two groups. The first group of 75 calves (conventionally fed (CF) group) was fed 3- 4 litres of milk replacer twice daily until weaning. The second group of 75 calves (transition milk (TM) group) were fed 3-4 litres twice daily of a pool of second and third milking transition milk for 5 days and were then fed 3- 4 litres of milk replacer twice daily until weaning. All calves were health scored using Wisconsin Madison health scoring (McGuirk et al., 2008) by one of two trained technicians every second day for the entire pre-weaning period. Farmers were provided with potassium sorbate and all TM was fed fresh or 'spiked' with 1% of 50% potassium sorbate before being stored in a large plastic drum for a maximum of 4 days at ambient temperatures before feeding. All first feed colostrum and TM (20 mL) was sampled daily at point of feeding and stored frozen at -20°C for subsequent testing. Calves were blood sampled weekly by jugular venipuncture by a veterinary clinician.

Laboratory analysis

The IgG concentration of the colostrum and TM was indirectly estimated (in duplicate) using a digital Brix refractometer. Serum IgG concentration was measured directly using radial immunodiffusion (Triple J) plates. Total aerobic bacteria count and coliform count for each colostrum and TM sample was also measured using Petrifilms™ following the protocol described by Morin et al., (2021).

Results

Preliminary results show that calves fed TM for 5 days performed better than milk replacer fed calves with lower mean faecal (p=0.05) and nasal scores (p=0.04), but there was no difference in cough and eye and ear scores between the two groups. Numerical differences were observed in time to first health score (≥1) for faecal (2.6 versus 2.2 days for TM and CF groups respectively) and respiratory disease events (15.8 versus 15 days for TM and CF groups respectively), but these were not statistically significant in the preliminary dataset. No differences in mean rectal temperature were observed between the two groups. Numerical differences were also observed in the weaning weights and average daily gains (ADG) between the two groups with TM calves weaned at 91.9kg with ADG of 0.76kg per calf per day and CF calves weaned at 90.9kg with ADG of 0.73kg per calf per day.

Mean total bacteria count (TBC) for day 1 colostrum was 113235.4 CFU/ml and mean coliform count (CC) was 3825.2 CF/ml, revealing that while TBC exceeded published thresholds of 100 000CFU/ml, CC was below the desired threshold of 10 000 CFU/ml. Mean TBC for TM was 142875.4 CFU/ml and mean CC was 4520.5 CFU/ml. There was no difference in serum IgG concentration between the calves in the two groups.

Conclusions

Preliminary results indicate health score and growth benefits to feeding TM to dairy calves for 5 days. First milking colostrum and TM was above published contamination thresholds for TBC (CFU/ml) indicating that farmer focus should be on basic colostrum storage and hygiene practices.

1089 - The concentration of copper in the liver over time with different methods and doses of injectable copper supplementation

Author: Gregory CHAMBERS, Matthew WELLS, Emma CUTTANCE

Objectives
Injectable products are commonly used to supplement copper to prevent deficiency and optimise productivity, but the amount of copper delivered to the cow and the durations of effect are unclear, so producers and veterinarians frequently plan supplementation based on assumptions. The objectives of this study were to determine liver copper concentrations of lactating dairy cows at six sampling events across a 2.5-month period before and after copper supplementation with three different injectable protocols compared to untreated control cows.
Material and methods
A randomised, controlled trial was run on two commercial dairy farms, chosen based on a sample of 15 randomly selected cows on each farm having mean liver copper concentrations of 150-1500 µmol/kg and the managers being willing to participate. Approximately 14 days prior to copper treatment, blood samples were collected from 109 randomly selected cows to measure plasma copper concentrations. On day 0, randomly selected cows were allocated to four treatment groups: 1) 100mg copper edetate subcutaneously [Copperguard®], 2) 200mg copper edetate subcutaneously [Copperguard®], 3) 75mg copper disodium EDTA subcutaneously [Multimin®], and 4) no treatment. Treatment group was randomly allocated within two age blocks (3-5 and 6-10 years). Liver biopsies were performed on days -7, 3, 13, 28, 42, 56, and 70 to determine liver copper concentrations. The primary outcome variable was the change in liver copper concentration compared to day -7. Exposure variables were treatment group, farm, cow age, cow age group, pre-trial plasma copper concentration, and day -7 liver copper concentration. The null hypothesis, that change in liver copper concentration after treatment is not associated with the treatment group, was tested by constructing a mixed multivariable linear regression model,
Results
There were 480 liver biopsy results from 80 cows. The treatment groups were balanced for age, plasma copper concentration prior to enrolment, and liver copper concentration at day -7. Farm A had lower liver copper concentrations at day -7 and a smaller overall change (across all treatment groups) after treatment than farm B, where liver copper concentrations increased in general across the study. Change in liver copper concentration was associated with the treatment group, study day, and farm, and a three-way interaction between them, and was negatively associated with liver copper concentration at day -7. There were significant differences between treatment groups only on farm A, where cows in group 2 had significantly higher increases in liver copper concentration than cows in a) group 4 at day 3, b) all other groups on day 14, c) groups 2 and 4 on day 28, and group 4 on day 42. There was substantial overlap between treatment groups on farm B, and even group 4 cows experienced an increase in liver copper concentrations at all time points compared to day -7. For each 10 µmol/kg increase in liver copper concentration at day -7, the change in liver copper at subsequent biopsies reduced by 1 µmol/kg.
Conclusions
A 200mg dose of copper edetate caused significant elevations in liver copper concentration compared to untreated cows for 42 days after treatment and compared to a 100mg dose of copper edetate and a 75 mg dose of copper disodium EDTA at one to two-time points each but this only occurred on one of the two farms, which had lower pre-treatment liver copper concentrations than the other farm. The effect of supplementation depended on pre-existing liver copper concentrations and unmeasured farm-level factors. Copper supplementation plans should therefore be made specific to each farm based on liver copper data and farm factors expected to affect copper reserves, such as dietary intake.

1373 - Ruminal fermentation and ruminal ammonia concentration in beef cattle supplemented with urea and urea-protected additives.

Author: Manuel Gonzalez Ronquillo, Lizbeth Robles Jimenez, Octavio Castelan Ortega, Sergio Angeles Campos, Emma Song, Angel Wu, Jorge Osorio Avalos

Objectives
An in vivo study was conducted to determine the effects of feeding feed grade urea (FGU) or slow-release urea (SRU) as a replacement for true protein supplements (soybean meal, SBM), and control diet (CTR, no N inclusion sources) in beef cattle diets to see if N-NH_3 concentration is reduced at the ruminal level during the first few hours, decreasing the risk of alkalosis.
Material and methods
An in vivo study was conducted to determine the effects of feeding FGU or SRU, as a substitute for true protein supplements (SBM), and control diet (CTR) in beef cattle diets fed as a single dose in the morning (0800 h) at the start of feeding to see if N-NH3 concentration is reduced at the rumen level during the first few hours, decreasing the risk of alkalosis. For this purpose, 5 beef cattle of 577 ±48.75 kg live weight, provided with ruminal cannula, using a conventional diet for beef cattle (concentrate/ corn silage, 60: 40 as DM) and the following treatments were used, Treatment 1, Control diet (Ctrl-without any N-additives), Treatment 2, Soybean meal (SBM) as reference protein source (Ctrl-SBM); Treatment 3, Urea-based diet, Treatment 4, SRU Optigen-based diet (replacing Urea); Treatment 5, SRU-Biuret based diet (replacing Urea); Treatment 6, Urea with the inclusion of HyUrit at 25% of DMI (Urea+ HyUrit). Randomly distributed to one of six experimental treatments, in a 5x6 incomplete Latin square design, treatments. The diet was isoenergetic and isoproteic with 11.4 MJ ME/kg DM and 14 % CP. Each experimental period lasted 30 d, of which 23 days were used for diet adaptation and five days for sample collection (intake and digestibility), the last two days were used for rumen dynamics testing (N-NH_3 and pH) at 0 (just before feeding), 0.5, 1, 2, 4, 6, 8, 12, and 24 h after feeding. Each beef cattle received each treatment once during each of the six periods.
Animals were weighed at the beginning and at the end of each experimental period. Additives were then offered daily in a single pulse dose at 08:00 h AM. Animal performance variables such as dry matter intake (DMI), digestibility, average daily weight gain (ADG), gain/feed ratio, chemical composition of diets, ruminal fermentation profile and N utilization were then considered.
Results
The average live weight of the animals was 568.7 ± 45 kg BW showing no differences between treatments ($P>0.05$), as well as the average daily weight gain (1.8 kg/d), were similar ($P<0.05$) between treatments. DM intake (14.32 kg/d), OM (12.6 kg/d), NDF (8.15 kg/d) and ADF (3.53 kg/d) were similar between treatments ($P>0.05$), however, significant differences ($P<0.001$) were observed for dry matter digestibility coefficient, with SBM, Optigen and Biuret being superior to HyUrit, and digestibility coefficient ($P>0.05$) for OM, FDN and ADF were similar between treatments.
It was found in the *in vivo* N-NH3 dynamics (mg/dl) of the rumen samples at different hours, HyUrit 25% shows the lowest N-NH3 concentrations ($P<0.001$), with respect to the rest of the treatments, on the contrary, the highest N-NH3 concentrations for hours 0.5, 1 and 2 were for feed grade Urea, Optigen and Biuret followed by control and SBM diets.
Conclusions
The addition of HyUrit25% inhibits the release of N-NH3 in the rumen at concentrations below 8 mg/dl, which diminish the risk of ammonia intoxication compared to the addition of feed grade urea, Optigen and Biuret.

1718 - Effect of Astragalus mollissimus on ruminal fermentation, methane emissions and performance of sheep´s

Author: Agustin Corral-Luna, Einar Vargas Bello Perez , Robin C. Anderson, Monserrath Felix-Portillo, Aleksandar K. Božić, Lorenzo Buenabad-Carrasco, Adrián O. Máynez-Pérez

Objectives
To determine the effect of dietary *Astragallus mollissimus* (which contains the nitrocompounds 3-nitro-propanol (3NPOH) and 3-nitro-propionic acid (3NPA)) CH_4 and volatile fatty acids production, animal performance and the presence of the nitrocompounds in blood of sheep.
Material and methods
In the present study, two experiments were carried out to determine CH_4 and volatile fatty acids production, animal performance and the presence of the nitrocompounds in blood. In experiment 1, four Pelibuey breed sheeps (BW 52.8 ± 6-05 kg) were assigned to 4x4 latin square arrangement. In experiment 2, 20 Dorper breed sheeps (BW 29.0 ± 3.0 kg) were randomized to five treatments.
Results
In both experiments the *A. mollissimus* was supplemented fully homogenized in diets consisting of oat hay 67% and concentrate 33%. The supplementation with different doses of nitrocompounds reduce (P≤ 0.05) the total gas and methane production. Methane was reduced 60% when 1 gr AM kg PV d was supplemented. In experiment 2, the no effects were observed in animal performance (feed consumption, average daily gain and feed conversion). Finally, no differences were observed for nitrocompounds in blood.
Conclusions
These results suggest that 3-NPA and 3-NPOH of biological sources possess viable characteristics to be considered as anti methanogenic alternatives.

1108 - Evaluation of brown and red sub-Antarctic macroalgae a as feed for ruminants

Author: Manuel Gonzalez Ronquillo, Lizbeth Robles Jimenez, Ashley Ulloa, Sergio Radic-Schilling, Ali Rivero, Pablo Gallardo Ojeda

Objectives

The inclusion of seaweeds in ruminant diets could potentially play a viable role in ruminant diets as a source of protein and energy.

Macroalgae can be used for several purposes including, for instance, human consumption, soil fertilizers and animal feed, due to their diverse range of metabolites (de Freitas et al. 2020). The objective of this study was to evaluate different macroalgae found in the Chilean sub-Antarctic Region, in terms of their chemical composition and in vitro ruminal fermentation.

Material and methods

Four seaweeds; Lessonia flavicans, Gigartina skottbergii, Ulva lactuca and Macrocystis pyrifera were used, which were collected in August 2023 in the intertidal, in Laredo Bay, Strait of Magellan (52°58" S. 70°48" W), the samples were dried (25ºC, 72 h) and ground to 1 mm diameter, and as a control a sample of alfalfa hay dried was used. All the algae and control were subsequently determined for chemical composition and gas production in vitro (Theodorou et al., 1994), for which 0.800 g of DM from each of the algae were incubated in triplicate in a glass bottle (125 mL), with 90 ml of buffer solution and 10 ml of rumen fluid from three sheep, and three incubation periods were carried out. Rumen fluid from three lambs (32 kg LW) were used as rumen fluid donors, coming from a Magallanes slaughterhouse, which had a diet based on 100% grazing forage (12 % CP, 2.3 Mcal ME/kg DM), the rumen fluid was extracted and filtered in triple cheese cloth gauze, and homogenized with CO_2. The bottles were incubated in a water bath at 39°C. Gas volume (ml gas/g DM) was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours of incubation using a pressure transducer (model 8804 HD). After the incubation period (96h), the dry matter disappearance (MSD96h mg/100mg), as well as Relative Gas Production (RGP, ml gas 96h)/(mg/100mg DMD 96h) were determined. Short chain fatty acids concentration (SCFA) according to Getachew et al. (2002) and Microbial Biomass Production (MBP) according to Blümmel et al. (1997). A completely randomized design was used, and a Tukey test was used when significant differences between treatments were observed ($P < 0.05$).

Results

Table 1 shows the chemical composition of the macroalgae. OM content, (g/kg) was higher for alfalfa hay ($P<0.0001$), followed by G. skottsbergi and lower for M. pyrifera, as for CP content ($P<0.0001$), it was higher for U. lactuca, followed by alfalfa hay and lower for G. skottsbergi. In vitro gas production (mL gas/ g DM) at 96h was lower ($P<0.001$) for G. skottsbergi, followed by M. pyrifera and L. flavicons, however G. skottsbergi and M. pyrifera show the highest Dig DM 96h (mg/100mg), being lower for U. lactuca.

Table 1. Chemical composition (g/kg DM) and In vitro rumen gas kinetics (mL gas/ g DM) and fermentation profile od different macro alga as apotential use in ruminat diets.

Item	G. skottsbergi	M. pyrifera	L. flavicons	U. lactuca	Alfalfa hay	SEM	P-value
OM,g/kg	744.63b	561.97e	693.77c	641.92d	899.13a	0.638	0.0001
CP,g/kg	86.00e	141.55c	111.86d	185.91a	154.50b	1.054	0.0001
EE, g/kg	17.68a	3.00d	1.65e	14.34b	8.57c	0.188	0.0001
ml gas/g DM							
6h	4.44c	7.78bc	4.42c	10.92b	16.31a	0.976	0.0001
12h	6.82d	12.45c	10.87cd	20.74b	40.41a	1.206	0.0001
24h	11.55d	14.68d	25.13c	33.42b	73.66a	1.621	0.0001
48h	20.97d	32.99cd	41.18c	58.56b	102.28a	3.142	0.0001
96h	25.88d	59.14c	48.83c	82.51b	118.28a	4.567	0.0001
DMD,mg/100mg	68.49a	67.62a	41.60b	14.72c	44.64b	0.765	0.0001
ME, Mj/kgDM	7.89e	11.27c	10.28d	15.07b	16.02a	0.101	0.0001
MCP, mg/g	679.80a	669.81a	404.98b	132.48c	413.95b	7.226	0.0001
SCFA	0.05d	0.06d	0.10c	0.14b	0.32a	0.007	0.0001
N-NH3, mg/dl	26.91a	21.05b	21.47b	30.67a	31.07a	2.293	0.0258

Conclusions

The inclusion of U. lactuca suggests a viable alternative in diets for sheep, while G. skottsbergii has a higher DMD in vitro, it has a lower nutritive value and fermentation at the rumen *in vitro* level. In vivo studies are suggested to evaluate its possible use in ruminant diets.

1443 - Comparative evaluation of near infrared spectroscopy portable and laboratory equipment in the nutritive analysis of corn silages

Author: Jorge Zegarra Paredes, Erick Diaz, Francisco San Emeterio

Objectives

The objective of this work was to perform a comparative evaluation of two near-infrared spectroscopy (NIRS) methods of forage analysis, one in the laboratory (NIRLab) and the other on-farm portable handheld (NIRPort) against a traditional wet chemistry analysis, and to statistically determine the precision of each in the nutritional analysis of corn silage samples produced in the irrigation areas of the coastal strip of Arequipa, Peru

Material and methods

Six samples of corn silage were collected from 06 different dairy farms in the coastal area of Arequipa, Peru. The collected samples were divided into 3 aliquots, 2 of them were desiccated and sent to USA for parallel analysis by wet chemistry and NIRS in a commercial laboratory [Rock River Laboratory Inc]. Wet chemistry (WC) analyses, as a reference method, were performed according to the Association of Official Analytical Chemists (AOAC) and [ANKOM®] methodologies, while the NIRLab analysis was performed on [Foss 5000®] and [Foss 2500®] equipment, with calibrations developed locally in Wisconsin, USA. The 3rd aliquot of the samples was scanned on a portable handheld NIRS device [PoliSPECNIR®] (NIRPort) at the place of origin. Statistical evaluation was performed using a paired *t* test between each NIR method and its corresponding WC analysis comparing each determined nutritional parameter.

Results

The comparison of the NIRLab method with WC on a dry basis, of 12 nutritional parameters, found no differences ($p > 0.05$) in dry matter (DM), crude protein (CP), insoluble protein in neutral detergent (NDIP) and acid detergent (ADIP), acid detergent fiber (ADF) and net energy of lactation (NE_L). Differences were found ($p < 0.05$) in neutral detergent fiber (NDF) (46.1 vs 49.4%), ethereal extract (EE) (2.4 vs 1.6%), ash (ASH) (7.1 vs 7.9%) lignin (LIG) (5.3 vs 4.0%) starch (STA) (22.6 vs 19.9%) and non-fibrous carbohydrates (NFC) (37.8 vs 34.5%) for NIRLab and WC respectively. The comparison of the NIRPort method with WC on a dry basis, of 08 nutritional parameters, did not find differences ($p > 0.05$) only in CP and ADF, but did find differences ($p<0.05$) in DM (31.2 vs 32.7%), NDF (47.6 vs 49.4%), EE (2.1 vs 1.6%), ASH (4.3 vs 7.9%), STA (21.9 vs 19.9%) and NFC (38.3 vs 34.5%) for NIRPort and WC respectively. When comparing the NIRLab and NIRPort methods, they only coincided in CP and ADF ($p > 0.05$) differing in the parameters DM, NDF, EE, ASH, STA and NFC ($p < 0.05$).

Conclusions

On average, the corn silages produced in the area had higher values of NDF (49.4%) and ASH (7.9%) and lower values of STA (19.9% and NFC (34.5%) than those recommended for good quality corn silages. The NIRLab method strongly underestimated the values of NDF and overestimated those of EE, STA and NFC. While the NIRPort method strongly underestimated the values of NDF and ASH and overestimated those of EE, STA and NFC. Both NIR analysis methods require calibration curves developed locally to be able to more accurately estimate the nutritional parameters of corn silages produced in the coastal strip of Arequipa, Perú

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1645 - Relationships Between Fecal Nutrient Content Milk Composition of Dairy Cows in Different Lactation Periods

Author: Hidir Gencoglu , Aykut Acar, Esra Abdullahoğlu Kara, Ozan Bilgen, Cantuğ Çağlıcan, Mustafa Demir, Cihat Koca

Objectives
Easy-to-use and accurate methods to help assess the nutrient digestibility of diets are still a major challenge for nutritionists. To date, fecal and milk properties have been evaluated separately, yet a possible relationship between them has not been investigated in depth. The aim of this study is to determine the relation between milk yield and milk composition of nutrient contents in feces of dairy cows in different yield of lactation dairy cows (high and low yield).
Material and methods
Therefore, we examined the fecal output and milk composition of 138 Holstein dairy cows with different lactation periods (High-Producing-HP vs. Low-Producing-LP, 44.9 vs. 26.1 L; 75.9 vs. 369.8 DIM; n = 46 and p<0.0001 for both) in Sahdem Dairy Farm (Bandırma, Turkey). For two consecutive days, the milk samples were taken from from the automatic milking machine (Delaval, Swedish), combined daily and placed in a single sample container. The fecal samples were obtained for two consecutive days and analyzed individually for each day, feces pH were measured immediately. Total mix ration (TMR) and concentrate feeds consumed daily by cows were recorded. Collected feces were combined daily, then dried and analyzed for fat, starch and ash. The averages of two-day results were compared with the PROC MIXED of SAS (HP vs. LP). Then, a Pearson's correlation between milk and fecal parameters was performed with the PROC CORR of SAS for each group. Also, the data from both groups were combined for a milk-controlled partial correlation.
Results
While fat-protein corrected milk and fat-corrected milk were different (p<0.0001), parity was similar between groups. Milk composition and properties were different (p<0.05), except MUN, lactose, and SCS between groups. Fecal compositions were different (p<0.05), except for pH and CP between groups. In milk-controlled partial correlation model, fecal DM had a negative and slight correlation with casein (r = -0.49), protein percentage and yields (r = -0.46, r = -0.42, respectively) of milk. The total number of correlated parameters (p<0.05) was higher in HP cows than the LP cows (13 vs. 1 in 147 combinations, respectively), especially between milk and fecal fat composition.
Conclusions
In conclusion, the fecal fat analysis can be a promising tool to better understand the digestibility of the dietary fat source in high-yielding early lactation cows rather than late lactation cows.

1573 - By pass Omega 3 fatty acids effect on reproductive and productive performance of dairy cattle

Author: Javier López Paredes, Monica Puyalto, Felipe Gayo, Juan Jose Mallo

Objectives

The objective of this study was to evaluate the effect of the supplementation with a rumen protected source of fatty acids n-3 (Omega 3, Hi-Flax® (NOREL ANIMAL NUTRITION)) on the productive and the reproductive performance of dairy cattle.

Material and methods

Data from one commercial farm located in Galicia (Spain) from January to September 2022 splitted in two periods, treatment (T_1) and control (T_2), were collected with a total of 995 records from 195 cows for productive parameters, and with 228 inseminations from 178 cows for reproductive parameters. The treatment consisted on the supplementation with 200 g/d of protected flaxseed (Hi-Flax), and the control diet consisted on an isoenergetic diet which included fat from Palm fatty acids (Hydrogenated PFAD). Analysed productive parameters were: milk yield (MY, kg/d), protein yield (PY, kg/d), fat yield (FY, kg/d), protein content (PP, %), fat content (FC, %), and somatic cells count (SCC); besides, for the reproductive evaluation, the success of artificial insemination (S.IA, dichotomic: 0, non-pregnancy in the insemination and 1, pregnancy in the insemination) and the number of insemination (N IA) until pregnancy were recorded.

Data were analysed using *lmer R package* from *R software* (R Core Team, 2021) through generalized linear mixed models' effects with repeated measures for productive parameters, including as fixed effects the number of parities, the days in milk (as covariate) and the treatment (T_1, Hi-Flax and T_2, control) and as random effect (permanent effect) the cow. In the case of SCC, a logarithmic transformation was performed in order to accomplish normality. For S.IA, general linear mixed model was performed assuming a Binomial distribution for the response variable, where the number of parities, and the number of insemination and treatment were included as fixed effects and the cow as permanent random effect. For the N.IA, generalized mixed model with number of parity and treatment were included as fixed effects (non-repeated measures model).

Results

Results showed an improvement of the productivity of the cows in T_1 with an increase in 0.863 kg (s.e =0.363, $p<0.05$), in 0.045 kg (s.e =0.016, $p<0.05$), and 0.035 kg (s.e =0.012, $p<0.05$), for MY, FY, and PY, respectively for T_1 comparing with T_2. Non-significant effects were observed in the content of fat and protein in milk (FP and PP, %) and a reduction of 20% of SCC were estimated (s.e =0.05, $p<0.05$), involving a greater status in health and welfare of the cows, and in addition, a reduction of the occurrence of mastitis, one of the most economic important diseases in dairy cattle. Furthermore, a higher value of S.IA was observed for T_1 of 58%, with an ODDs ratio of 1.79 ($p=0.07$). Finally, the N.IA was 0.63 lower for T_1 ($p=0.074$). Subsequently, these reproductive parameters will involve a reduction on the calving interval and open days of the cow, with the subsequent impact on the savings for the farmers.

Conclusions

In conclusion, this study showed the benefits of the supplementation with by-pass omega 3, promoting a greater reproductive and productive performance and hence, an enhancement of the cow and farm profitability, reducing the non-productive periods and improving the productivity and health status of the cows.

1559 - The effect of feeding a mineral binder during the close-up period to multiparous Jersey cows on postpartum blood minerals – A randomized field trial

Author: Anay Ravelo, Mark Thomas, Matias Stangaferro, Luciano Caixeta, Rita Couto Serrenho

Objectives
Negative Dietary Cation-Anion Difference (DCAD) close-up programs reduce the risk of hypocalcemia and improve performance of multiparous cows. However, because of the need of frequent urine pH monitoring with concomitant ration adjustments, negative DCAD programs can be challenging. Zeolites, added to the TMR and used as a mineral binder, can be offered to control hypocalcemia without the need to monitor the acidification level of close-up cows. The objective of this study was to assess the effect of supplementing a close-up diet with a commercially available mineral binder (BINDER; target of 288 g/d sodium aluminosilicate, X-Zelit, Protetka) on postpartum blood minerals compared with a negative DCAD diet (group targeted urine pH: 5.8-6.3). We hypothesized that close-up cows fed BINDER would have greater blood Ca concentration postpartum than close-up cows fed a negative DCAD.
Material and methods
This randomized field study was performed in one commercial dairy farm located in Tulare, California (3,400 milking cows). From OCT 2022 to JUN 2023, pregnant Jersey cows entering their ≥2 lactation (n = 1,511 cows) were assigned to 1 of 2 dietary treatment groups, corresponding to two close-up pens. During the enrollment period, cows were moved to the close-up pens weekly (21d before the expected parturition). Close-up pens were replicated during 6 periods (every 4 weeks, the close-up cows and the dietary treatment fed to each close-up pen were switched). Group urine pH was assessed once per week in each group and dietary adjustments were performed to the DCAD diet accordingly. The dietary treatment was applied to the close-up pens and the outcomes were assessed at the cow-level. In a subset sample of cows, blood samples (0, 24, and 96 h postpartum) were collected (70 DCAD cows and 63 BINDER cows). All study periods were equally represented in this subset sample. The outcomes of interest were blood total Ca, P, Mg, Na, K, Na/K Ratio, Chloride, TCO_2, and Anion Gap. Mixed linear models accounting for repeated measures were built for each outcome of interest including dietary treatment (DCAD vs. BINDER), sampling time (0, 24, and 96 h), the interaction of treatment by sampling time, parity (2^{nd} vs. $>2^{nd}$) and days in close-up. A statistical significance was set at P <0.05; results are reported as LSM ± SE.
Results
The group urine pH of the DCAD and BINDER cows were 6.1 ± 0.1 and 8.1 ± 0.02, respectively. Of the 133 cows, 78 (59%) entered their third or greater parity. We did not detect a treatment effect on Na, K, Na/K Ratio, or AnionGap. An interaction of treatment × sampling time was detected in the Ca, P, Mg, Chloride, and TCO_2 models. Compared with DCAD group, Ca concentrations were greater in the BINDER group at 0 h [1.82 ± 0.02 vs. 2.05 ± 0.03 mmol/L] and 24 h [1.78 ± 0.02 vs. 1.95 ± 0.03 mmol/L]; we did not detect Ca differences between treatments at 96 h. The concentration of P and Mg were lower in the BINDER cows at 0h [P: 1.52 vs. 0.79 ± 0.05 mmol/L mmol/L; Mg: 0.96 vs. 0.78 ± 0.02 mmol/L] and 24 h [P: 1.69 vs. 1.22 ± 0.05 mmol/L; Mg: 0.95 vs. 0.79 ± 0.02 mmol/L] than in the DCAD cows; we did not detect P and Mg differences between treatments at 96 h. At 0 h, DCAD cows had lower blood TCO_2 [23.3 ± 0.28 vs. 25.7 ± 0.29 mmol/L] and greater Chloride concentrations [103.2 ± 0.39 vs. 100.8 ± 0.41 mmol/L] than BINDER cows; no differences detected at 24 and 96 h.
Conclusions
Feeding a mineral binder to close-up parous jersey cows yielded greater blood total Ca concentration in the immediate postpartum when compared to a tightly controlled negative DCAD strategy. Health and performance results are expected to conclude on the economics between both strategies.

1213 - Biochemical effects of preventive protocols for ketosis during the transition period in dairy cows

Author: Anastasia Lisuzzo, Alessio Valenza, Andrea Biancucci, Alex Bach, Matteo Gianesella, Enrico Fiore

Objectives

Metabolic adaptation failure during the transition period is characterized by excessive lipomobilization and metabolic stress leading to increased ketone bodies levels, as β-hydroxybutyrate (BHB). Hyperketonemia or subclinical ketosis is a major metabolic disease in dairy cows negatively affecting animals' health. Fatty acid oxidation and export capacity of the liver, and the proper functioning of gluconeogenesis and Krebs cycle through their precursors (especially propionate, gluconeogenic amino acids (AA), vitamins and co-factors) are essential to reduce the risk of hyperketonemia during post-partum period. The aim of this study was to investigate the effect on biochemical profiles associated with different preventive protocols for ketosis in dairy cows.

Material and methods

594 Holstein-Friesian dairy cows were randomly selected from the same farm at the begging of the dry period and equally divided in four groups following a time-series design (protocol number of ethical approvals 204359/2023):

i. CTR- control group without preventive treatment;

ii. CPP- complete preventive protocol group with two treatments of AA, inositol, and cyanocobalamin at 15 and 12d during the pre-partum (pre-P), 2 mL/10 kg of BW (IM), Bograss and seven treatment with acetyl-methionine, α-Lipoic acid and cyanocobalamin, 20 mL/animal, Erbacolina Plus each other day from calving to 12d post-partum (PP);

iii. SPP- simplified preventive protocol group received two treatments of Bograss with previously dosage and Erbacolina Plus (70 mL/animal per treatment) at 12d pre-P and 6d PP);

iv. MON- monensin group received one monensin bolus (35.2 g/animal, Kexxtone) at 21d pre-P.

All groups received the same diet. Blood sampling was performed from the coccygeal vein at -21 and -7d pre-P, at the day of calving, and at +7, +14, +28, and +55d PP.

Serum NEFA and BHB concentrations were assessed in all animals. Biochemical analyses were performed on 45 animals per group (30 multiparous and 15 primiparous). Liver functionality index (LFI) was calculated based on albumin, total bilirubin, and cholesterol values at +7 and +28d PP. Differences in biochemical parameters were assessed with a linear mixed-effects model. A post hoc pairwise comparison was performed using Bonferroni correction. A $p\text{-}value<0.05$ was considered significant, whereas a $0.05\leq p\text{-}value\leq 0.10$ was considered a trend.

Results

Lower NEFA level curves were found in CPP and SPP compared to CTR and MON during PP, but increases were noted in primiparous on CPP and SPP at the day of calving compared to CTR cows. Serum BHB concentration was generally lower in CPP and SPP compared to CTR and MON between 14 and 55d PP, with an hyperketonemic state in primiparous cows on CTR at 28d PP and pluriparous cows at 14d PP. All treated groups had an improvement in serum glucose at calving compared to CTR, which incurred hypoglycemia at 7d PP. Serum albumin concentrations decreased from 7d pre-P in CTR, 7d PP in CPP and from 14d PP in MON, while increased in SPP with the greatest level at 14, 28, and 55d PP. Serum urea was lower in CPP and SPP at 7 and 14d PP compared to CTR. Serum AST levels were within the physiological range, but lower

concentrations were noted in CPP and SPP from calving to 14d PP compared to CTR and MON, and at 28d PP compared to only CTR. Serum GGT was greater in the MON from calving to 55d PP with the lowest level in CPP at 28 and 55d PP. Serum ALP concentration was greater in CPP and SPP from 7d AP to 55d PP compared to CTR and MON with greater level of Ca from calving to 14d PP. Furthermore, serum Cl and Na were greater in the same groups around calving. Lastly, SPP cows had the greatest LFI with no differences found between CTR and CPP cows.

Conclusions

The preventive protocols CPP and SPP reduced lipomobilization, metabolic stress, and protein catabolism, and improved liver health status and calcium mobilization. In addition, SPP resulted in a better liver functionality and support to albumin production. In conclusion, providing precursors for gluconeogenesis and Krebs cycle had a better effect on the animal biochemical profiles, particularly by providing precursors in both pre and post-partum.

1785 - Enhanced Creep Feeding with Calcium Propionate: Boosting Ruminal Papillae Development, Performance, and Metabolomic Meat Features in Lambs

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Objectives
This study aimed to assess the impact of incorporating Calcium Propionate (CaPr) in creep feeding on various aspects of lamb growth performance, including ruminal papillae development, carcass characteristics, meat metabolomics, and mRNA expression of G protein-coupled receptor 41 (GPR-41) and G protein-coupled receptor 43 (GPR-43).
Material and methods
Twenty-four Rambouillet baby lambs (initial BW 6.61 ± 1.49 kg) were randomly allocated to one of the following treatments: 1) control, without creep feeding, only maternal milk (CONT); 2) Creep feeding (basis) (CF); and 3) Creep feeding plus CaPr (50 g/kg DM basis) (CFCaPr). Following 52 days of feeding on these diets, lambs were slaughtered, and various measurements were taken, including rumen characterization, carcass measurements and meat samples from *longissimus dorsi*. The data were analyzed as a complete randomized design, and means were compared using the Tukey test. Metabolomic analysis of meat metabolites was conducted using MetaboAnalyst 5.0.
Results
Creep feeding resulted in increased ($P \leq 0.05$) average daily gain and final body weight. Animals receiving creep feeding with added CaPr showed higher ($P \leq 0.05$) hot carcass and cold carcass weights and a decrease ($P \leq 0.05$) in pH at 45 minutes and pH at 24 hours, compared to the effects of creep feeding alone. Rumen characteristics indicated increased ($P \leq 0.05$) reticulum-rumen weight for animals in the CFCaPr treatment. Papillae width and epithelium thickness were also influenced ($P \leq 0.05$) by CFCaPr treatment, with CF papillae being larger ($P \leq 0.05$) than the other treatments. Short-chain fatty acid receptors (GPR41 and GPR43) in ruminal tissue decrease ($P \leq 0.05$) in the CF and CFCaPr treatment compared to CONT. Metabolomic analysis revealed that using CaPr in creep feeding supplementation significantly affected ($P<0.05$) the biosynthesis of unsaturated fatty acids in the meat of supplemented baby lambs.
Conclusions
Incorporating CaPr in creep feeding is a sustainable strategy for enhancing ruminal development.

Reproduction

1622 - The influence of the uterine activity in the cows during postpartum period

Author: Zdzisław Gajewski, Maria Sady, Karolina Ferenc, Romuald Zabielski, Ewa Sady, Axel Wehrend

Objectives

The postpartum period in cattle refers to the time immediately following parturition (calving). During this period, various physiological changes occur in the reproductive tract, including the uterus. Uterine contractility plays a crucial role in the expulsion of fetal membranes, involution of the uterus, and prevention of postpartum complications. Several factors influence uterine contractility in cattle during the postpartum period, and drugs can be used to modulate these processes. Understanding and monitoring uterine activity in both physiological and pathological states are essential for managing reproductive health in cattle. Techniques such as ultrasonography, electromyography, and hormonal assays can be employed to assess uterine function and diagnose reproductive disorders in bovines. Additionally, proper nutrition, hygiene, and veterinary care are crucial for maintaining optimal uterine health in cattle.

The objective of this study was to control and determine uterine motility and the measuring of the influence of different drugs on the uterus with EMG and USG methods during the postpartum period in cattle.

Material and methods

In the experiment were 14 H-F cows 4-7 years old, 600-800 kg b.w. were used for the measuring of USG and EMG uterine activity. During the surgery in the three different locations in the myometrium of the uterus 3 electrodes were implanted as described elsewhere (Gajewski and Faundez, 1992). Electrical activity was recorded using a 6 channel pen-recorder, of sensitivity 1 V cm -1 connected to a biological amplifier WSB-4 (amplification 2000 x) as described earlier (Gajewski and Faundez, 1992). Frequency band was 2-100 Hz. For the analysis of the EMG activity were used: duration of the electrical activity bursts (APB) in sec., myoelectrical index (MI= d1/T x 100 - d1 being the sum of duration of the bursts recorded during a session of T sec.) and the number of APB in a 10 min. period. The ultrasound examinations were performed daily with real time B-mode scanner equipped with a 5.0 MHz linear-array transducer (Fa. Esaote).

Results

The application of oxytocin (Oxytocin, Fa. Vet-Agro) showed a significant increase in EMG activity (amplitude, frequency, and duration of the APBs). The same result was observed after the application of PGF 2α (Dynolitic, Fa. Zoetis) on the myoelectrical uterus activity, which was characterized by the increase of duration of the myometrium activity (both uterine horns and corpus uteri). The application of Carbotocinum (Hypophysin LA, Fa. Veyx-Pharma) increase the uterine motility (in amplitude and frequency of EMG uterine activity). The USG measuring showed the decreasing of the uterus horn diameter and the length of the uterus. The hormonal analysis showed at the beginning of post partum presented the decreasing of P4 from 0,7 ng/ml till and < 0,2 ng/ml amounts. The level of E2 decrease from over 150-200 pg./ml on the day of parturition to 10-15 pg./ml on 14 days postpartum.

Conclusions

The results showed that the understanding and monitoring of uterine activity in both physiological and pathological states are essential for managing reproductive health in cattle. Techniques such as ultrasonography, electromyography used to measure the influence of different drugs like PGF 2α, oxytocin, carbotocin etc., and hormonal assays can be employed to assess uterine function and diagnose reproductive disorders in bovines. Additionally, proper nutrition, hygiene, and veterinary care are crucial for maintaining optimal uterine health in cattle

1418 - Pregnancy Associated Glycoprotein Carryover in Individual Milking Robots

Author: RAFAEL PAIVA , Ky Pohler, Juan Piñeiro

Objectives

The objective of this study is to determine, identify and measure the level of PAG carryover in milk samples using the [Lely sampler for the Astronaut robot] using a commercial sample-based pregnancy test.

Material and methods

The dairy is in Archer County in North-West Texas, USA, milking 1,100 cows in a force ventilated barn, with 4 pens and twelve robots, three in each pen, two in tandem and one satellite robot in a management pen. Every two weeks, samples from eligible cows (day 28 to 42 after AI) will be collected by hand with the cows locked in the management pen. A list of cows to be tested is created in the software and sent to the robots. The same day, the cows will exit the management pen thru the satellite robot, a sample will be collected automatically by the robot sampler. The robot software provides a list of cows sampled and the tube location in the sampler, tubes are identified and collected at the end of the day, placed in a box, and sent to the laboratory.

The Sampler is a removable and external sampling device for the [Lely Astronaut milking robot. Model ARSD L90, type 05]. When connected, the Sampler can automatically collect up to 90 milk samples. (N.V.)

The private laboratory will run the [IDEXX Alertys® Milk Pregnancy Test] following the manufacturer insert and send the results for analysis.

A Receiving Operating Characteristic Curve (ROC curve) was built to determine sensitivity (Se), Specificity (Sp), Negative Predicted Value (NPV) and Positive Predicted Value (PPV). A Spearman's Coefficient of Rank test was performed with a 95 % Confidence Interval.

Open cows that came into the robot after a pregnant cow were identified and results followed to determine if there was carryover.

Results

Se and Sp, as well as NPV and PPV are 98 % when manual sample collection is used as gold standard.

The correlation found with a 95 % CI, is 0.786 (0.699 to 0.849) ($P<0.0001$).

In cases where open cows came into the robot after a pregnant cow, there was only 2 % of carryover identified.

Conclusions

There is a strong positive correlation of 0.786 (0.699 to 0.849) between the milk samples collected manually and the ones collected by the robot samples with a 95 5 CI and $P<0.0001$. Samples collected by hand will have the same performance than samples collected by the robot sampler.

Se and Sp, NPV and PPV are 98 %, this show only a 2 % of false positives and false negatives which is very close to what the test manufacturer discloses in his performance reports.

When open cows are milked after a pregnant cow there is only 2 % of carryover, 98 % will have a correct pregnancy diagnose.

The [Lely sampler for the Astronaut robot] can be used with a strong confidence on the results for open and pregnant cows.

In robotic dairies with the [Lely Astronaut], producers can use the robot sampler to collect milk samples for pregnancy determination with strong confidence on the results, this will allow to do a noninvasive pregnancy check at early stages of gestation, with less stress, better comfort, and animal welfare, that, based on other research, will increase the herd milk production.

More research in different robots' setup should be performed for a better understanding of carryover in different brands and configurations.

1354 - Pregnancy evaluation with a point-of-care pregnancy test in dairy cattle

Author: Zoltán Szelényi, Ildikó Lipthay, Attila Sánta, Lea Lénárt, Atilla Répási, Szenci Ottó

Objectives
Primiparous and multiparous dairy cattle were evaluated for pregnancy using both transrectal ultrasonography and a point-of-care pregnancy test (Alertys OnFarm Test), which measures pregnancy-associated glycoproteins through lateral diffusion, between Days 28–34 of pregnancy results were compared. This study aimed to evaluate the usefulness of a point-of-care pregnancy test in dairy cows and non-lactating heifers to accurately identify pregnant animals, which may help reduce professionals' workload.
Material and methods
A total of 637 animals were included in this study. Pregnancy was confirmed via manual palpation between Days 57–64. Data on parity, calving, and time of artificial insemination (AI) were also collected and evaluated. None of the animals included in the study were tested more than once for early pregnancy. Pregnancy on transrectal ultrasonography was determined as the presence of an embryo with a heartbeat, fluid in the uterine horn, and at least one corpus luteum (either cavitary or compact). Only animals with a precise diagnosis of the mentioned four criteria were included in the study.
The veterinary workload was also evaluated. The average length of the scanning event was defined as the duration of pregnancy examination event at the farm: on Farm A, 60min was calculated as the time load for one pregnancy examination event, whereas on Farms T and Z, it was 120min. Using the combined method of pregnancy testing, animals were first tested with the lateral diffusion test; then, the test-negative animals were scanned again.
Results
Overall the accuracy of the lateral diffusion test was 93.1% with 98.9% sensitivity, 88.7 % specificity, 86.8 % positive predictive value, and 99.1% negative predictive value. In heifers, the Alertys OnFarm Test had 100% sensitivity and 81.6% specificity. In contrast, the test had a sensitivity and specificity of 98.5 and 89.5%, respectively for multiparous cows. The pregnancy loss between early diagnosis and confirmation increased with parity. Heifers suffered losses as low as 2.6%, whereas animals in the third parity had significantly more losses (17.9%). Season also affected losses with spring pregnancy losses being considerably higher than autumn losses. Regarding to veterinarian workload using theoreticly the combined method the number of scanned animals was reduced. The results ranged between 32-74% on farms.
Conclusions
When comparing other results with ours, it is visible that sensitivity results are now close to ideal in several reports, while specificity results in the different studies are always somewhat lower. These accuracy results carry a common consequence, that pregnancy testing results can be distributed at farm level. As we apply different reproduction protocols for nulliparous and multiparous animals, similarly there is a possible option to distribute pregnancy testing as well. Notably, 10% of the test-positive animals showed a negative result on ultrasonography. In clinical circumstances, the accuracy of a preg- nancy result is greatly influenced by two major factors: the accuracy of the pregnancy test and pregnancy losses. In this respect, the endeavour to achieve high sensitivity means eliminating possible false negative cases generated by the pregnancy test itself. In our study, the aggregated data evaluation showed excellent, but not absolute, high sensitivity, meaning that in each sample population, there will always be approximately 0.5–1% of the examined animals with false negative diagnoses when the point-of-care test is used.When data were distributed to nulli- and multiparous animals, the sensitivity increased to up to 100% in our study, meaning that the initial early pregnancy diagnosis can be

per- formed via this point-of-care test in case of heifers. Thus, pregnancy test results can be evaluated according to parity, and provide accurate re- sults for non-lactating heifers.
We concluded that veterinary workload could be reduced by using the point-of-care test. In our study, the theoretical reduction in the physical workload of the professionals decreased the number of animals undergoing TRUS by at least one-third. This way the combination of pregnancy determination methods gives a tool for the reduction of hazards during workload in the future. Our results have also highlighted that no pregnancy diagnosis ex- amination event can be omitted; however, there is a wide range in the reduction percentage of the veterinarian workload between farms. However, farm-level differences may have affected the results of the present study.
Reference: https://doi.org/10.1016/j.theriogenology.2023.10.014.

1434 - Effect of a targeted reproductive management program based on automated estrus alerts during the voluntary waiting period on reproductive performance of lactating dairy cows

Author: Ana Laura Laplacette , Clara Rial, Gloria Stephanie Magaña Baños, Jose Alberto Garcia Escalera, Siddartha Torres, Allison Kerwin, Julio Omar Giordano

Objectives
The objective of this randomized controlled experiment was to evaluate the effect of a targeted reproductive management (**TRM**) program that prioritized insemination at detected estrus (**AIE**) based on automated estrus alerts (**AEA**) during the VWP on the reproductive performance of lactating dairy cows. This program was expected to optimize first service management by providing different amounts of time for insemination at detected estrus to cows with or without AEA during the VWP which are more and less likely to express estrus immediately after the end of the VWP, respectively.
Material and methods
Lactating Holstein cows (n = 1,260) from a commercial dairy farm in Mexico were blocked by parity and semen used for first AI (Holstein vs. beef) and randomly assigned to a targeted reproductive management program designed to prioritize first service after detection of estrus by automated estrus alerts (**TP-AIE**; n = 632) or a program that used all-timed AI (**TAI**) and an extended VWP (**ALL-TAI**; n = 628). Automated estrus alerts recorded by an automated behavior monitoring system (SenseHub Dairy, MSD Salud Animal) from 15 to 49 days in milk (**DIM**) were used to create groups of cows with at least one (**E-VWP**) or no AEA (**NE-VWP**) during the voluntary waiting period (**VWP**). After a 50 d VWP, cows in the TP-AIE treatment and E-VWP group (n = 346) were eligible for AIE for 28 d whereas cows in the NE-VWP group (n = 233) were eligible for AIE for 14 d. Cows not inseminated at detected estrus received TAI after Ovsynch with progesterone supplementation at 90 ± 3 or 76 ± 3 DIM if in the E-VWP and NE-VWP group, respectively. Cows in the ALL-TAI treatment received TAI at 76 ± 3 DIM after a Double-Ovsynch protocol. Binary data were analyzed using logistic regression and time to pregnancy with Cox's regression. Models included parity, treatment, estrus group, and the treatment by estrus group interaction as fixed effects. Season of parturition, calving ease score, health disorders occurrence by 14 DIM or 15 to 49 DIM, Daughter Pregnancy Rate, and all 2-way interactions with treatment were offered as covariates.
Results
The proportion of cows with AEA during the VWP did not differ ($P = 0.41$) for ALL-TAI (56.1%) and TP-AIE (52.8%). As expected, due to their greater likelihood to express estrus and more time available for AIE, more cows ($P < 0.001$) in the E-VWP (83.3%) than the NE-VWP (45.0%) group within the TP-AIE treatment received AIE. First service DIM were reduced ($P < 0.001$) for the TP-AIE (69.0 ± 0.7) than the ALL-TAI (75.7 ± 0.8). The hazard of pregnancy (HR=1.0; 0.9-1.2) did not differ between treatments ($P = 0.97$) and median days to pregnancy were 102 for the ALL-TAI and 107 for the TP-AIE treatment. The proportion of cows pregnant at 150 DIM did not differ ($P = 0.33$) for the ALL-TAI (59.1%) and the TP-AIE (56.0%) treatment. First service pregnancies per AI (**P/AI**) were greater ($P < 0.001$) for the ALL-TAI (42.3%) than for the TP-AIE (29.0%). For second service, there were no differences in the proportion of cows AIE (ALL-TAI = 59.8% vs TP-AIE = 65.5%, $P = 0.21$) and P/AI (ALL-TAI = 40.1% vs TP-AIE = 39.9%, $P = 0.84$), but average DIM at second service was earlier (TP-AIE = 101.1 ± 1.6 DIM vs. ALL-TAI = 112.7 ± 1.7 DIM; $P < 0.001$). A greater number of services were performed before 150 DIM for the TP-AIE (2.1 ± 0.1) than the ALL-TAI treatment (1.8 ± 0.1; $P = 0.001$).
Conclusions
We conclude that a targeted reproductive management program designed to optimize the use of AIE with an automated behavior monitoring system for detection of estrus through the use

of estrus alerts collected during the VWP led to similar reproductive performance by 150 d after calving than a program that used all TAI after a fertility protocol and had an extended VWP. Despite the fewer P/AI at first service because of earlier DIM at first insemination, the TRM program was effective because more than 50% of the cows had estrus alerts during the VWP, days to first service were optimized through the use of AEA, and the use of a shorter VWP provided earlier and more opportunities for reinsemination.

1433 - Association between estrus detected by an automated behavior monitoring system during the VWP with physiological outcomes and reproductive performance of lactating dairy cows

Author: Ana Laura Laplacette , Clara Rial, Gloria Stephanie Magaña Baños, Jose Alberto Garcia Escalera, Siddartha Torres, Allison Kerwin, Julio Omar Giordano

Objectives

The objectives were to evaluate the association between estrus detected by automated estrus alerts (**AEA**) during the voluntary waiting period (**VWP**) with cow physiological features and reproductive performance for lactating dairy cows submitted to management programs that prioritized insemination at detected estrus (**AIE**) or used all- timed AI (**TAI**) and extended VWP for first service.

Material and methods

Lactating Holstein cows (n=1,260) at a farm in Mexico were detected in estrus with an automated behavior monitoring system (SenseHub Monitoring Neck Tag, MSD Salud Animal) from 15 to 49 days in milk (**DIM**) to record AEA. Body condition score (**BCS**; 1 to 5 scale with 0.25-point increments) was assigned upon entry in the close-up pen, at calving, and at 6, 34, and 46 DIM. Cows were grouped based on BCS change [gain, no change, and loss] between calving and 46 DIM. Locomotion scores (**LS**) were recorded at 34 and 46 DIM as 1=normal, 2=mildly lame, 3=moderately lame, 4=lame, 5=severely lame. Lameness was defined as LS ≥3 at least once. Cows were grouped in a high, medium, and low Daughter Pregnancy Rate (**DPR**) group. In a subset of cows (n=343), vaginal discharge scores (**VDS**) were assigned at 34 and 46 DIM based on: 1 = clear mucus, 2 = mucus with flecks of pus, 3 = <50% of purulent material, 4 = ≥50% purulent material, 5 = score 4 with stench. Purulent vaginal discharge (**PVD**) was defined as VDS ≥3 at least once.

At 6 DIM, cows were randomly allocated to a program that prioritized AIE detected by AEA (**TP-AIE**; n=632) or a program that used all-TAI and an extended VWP (**ALL-TAI**; n=628) for first service. After a 50 d VWP, cows in the TP-AIE with AEA during the VWP (n=346) were eligible for AIE for 28 d whereas cows no AEA during the VWP (n=233) were eligible for AIE for 14 d. Cows not AIE received TAI after Ovsynch with progesterone supplementation. Cows in ALL-TAI received TAI at 76±3 DIM after a Double-Ovsynch protocol. Binary data were analyzed by logistic regression, time to pregnancy with Cox's regression, and continuous data by ANOVA.

Results

Overall, 58.7% of cows had an AEA during the VWP. More cows had AEA during the VWP if calved during the cold than the warm season (63.2 vs 56.2%, *P*=0.04), if in the high rather than the low or medium DPR groups (64.9% vs. 45.9 or 52.2%, *P*<0.001), if had no health disorders before 14 DIM (61.9 vs 41.9%, *P*<0.001) and there was a tendency for more primiparous than multiparous cows (61.8 vs. 56.8%, *P* = 0.06) to have AEA. Cows in the estrus group had greater BCS from 6 to 46 DIM than cows in the non-estrus group (*P*<0.001). Overall, 84.2%, 12.9%, and 2.9% of the cows lost, maintained, and gained BCS. Cows that maintained BCS were more likely to have AEA during the VWP (72.1%, *P*=0.01) than cows that lost (58.3%) or gained BCS (53.5%). More (*P*<0.001) cows without (62.0%) than with (44.9%) lameness (17.8% had lameness) and more cows without (64.6%) than with PVD (45.4%, *P*=0.003) had AEA recorded during the VWP (23.0% had PVD).

Cows in the TP-AIE program with AEA during the VWP were more likely to receive AIE for first service (83.3 than 45.0%, *P*<0.001). Regardless of treatment, cows with AEA during the VWP had more P/AI to first service (42.5 vs 28.9%, *P*=0.001), fewer pregnancy losses (9.1 vs. 18.8%, *P*<0.01), were more likely to receive second service by AIE (74.7 vs 48.9%, *P*<0.001), had more P/AI at second service (46.1 vs. 35.8%, *P*=0.01), had greater pregnancy rate (HR=1.6; 1.4-1.9,

P<0.001), and more were pregnant by 150 DIM (67.4 vs 47.0%, *P*<0.001) compared with cows without AEA during the VWP.

Conclusions

Absence of PVD, lameness and health disorders through the VWP, higher BCS after calving and maintaining BCS during the VWP, high DPR, and primiparity were positively associated with the likelihood of having AEA during the VWP. Multiple physiological mechanisms and cow features could contribute to explain the improved reproductive performance of cows with AEA during the VWP regardless of the type of program used for submission to first service.

1407 - Predicting the Reproductive Performance of Transition Cows on Automatic Milking Systems

Author: Fergus Hannon , Martin Green, Laura Randall, Luke O'Grady

Objectives

Targeted Reproductive Management (TRM) aims to improve the fertility efficiency of the dairy herd by applying bespoke group level management based on expected reproductive performance. To apply TRM, models capable of accurately predicting reproductive performance are required. The capability of Automatic Milking Systems (AMS) to collect a wide range of data points reflective of the animal's physiological status, means they provide a unique opportunity to develop such models.

The objective of this study was first to investigate the relationship between data collected by automatic milking systems during the first 3 weeks post-calving and subsequent reproductive performance. And second, to assess the utility of this data for the prediction of reproductive performance using machine learning models.

Material and methods

Thirty-six commercial AMS herds from the United Kingdom and Republic of Ireland were enrolled in this retrospective cohort study. Two binary outcomes reflective of reproductive performance were investigated. The first was the expression of oestrus (EO) between days 22 and 65 post-calving. The second was conception to first insemination (CFI) between days 22 and 80 post-calving.

AMS data describing the daily mean recordings for milk quantity, milk quality as well as visit behaviour and concentrate intake over the first 3 weeks post-calving were assessed. Ten variables were engineered based on their biological plausibility to reflect physiological status during this period. These were used in mixed effect logistic regression models together with selected interaction terms, to investigate the relationship between physiological status in early lactation and reproductive performance. Goodness-of-fit for the final logistic models was assessed by sensitivity and specificity of classification following backwards prediction.

To assess the accuracy with which AMS data could predict each outcome, two predictive models were constructed and external validated. For each outcome, all 10 features were offered to a random forest recursive feature elimination model to evaluate the relative importance of each predictor variable. Retained variables were brought forward in a final random forest model trained to maximise the area under the receiver operator curve (AUC-ROC) over 5-fold cross validation repeated 10 times. The final predictive models were evaluated by assessing sensitivity, specificity and AUC-ROC following external validation.

Results

Oestrus Expression

Inferential Modelling:

The final data set for EO consisted of 9,741 lactations from 25 herds. Fat:Protein Ratio, Milk Conductivity, Conductivity Alerts and Milk Yield, were all negatively associated with the odds of oestrus expression. Both Milk Protein and Milking Visits recorded a positive association.

Prediction Modelling:

Six variables: Milk Yield, Concentrate Intake, Milk Protein, Refusals, Conductivity and Milking Visits were retained for use in the final predictive model. Following external validation, a sensitivity of 55%, specificity of 67% and an AUC-ROC 0.65 were achieved.

Conception to First Insemination

Inferential Modelling:

The final data for CFI set consisted of 8,653 lactations from 23 herds. In the final inferential model, Fat:Protein Ratio, Milk Yield and the interaction term Milk Yield X Concentrate Intake

had a negative association with the odds of conception to first insemination. Concentrate Intake recorded a positive association.

Predictive Modelling:

7 variables were retained in the final predictive model, Concentrate Intake, Milk Yield, Milk Conductivity, Delta Yield, Milking Visits, Fat:Protein Ratio and Refusals. Classification on the external data set was performed with a sensitivity of 10%, specificity of 88% and an AUC-ROC of 0.52.

Conclusions

The success with which the dairy cow navigates the first 3 weeks post-calving is crucial to her subsequent fertility. These results demonstrate the association between AMS data collected over this time and reproductive performance. However, despite these associations the predictive ability of this data is lacking. This highlights the crucial difference between significant statistical association and predictive power.

The outcomes of interest were selected for this study based on the ease with which they can be incorporated into a TRM stragety. For instance, animals likely to conceive to first insemination can be preferentially targeted with sexed semen. However, the sensitivity and specificity achieved in the predictive models reported here are not sufficient to facilitate implementation of TRM. The potential for cow level sensor data such as rumination and activity to improve the predictive power of the models reported here warrants investigation.

1371 - Evaluation of a needle-free injection system for administration of cloprostenol for luteolysis in lactating dairy cows

Author: Alyssa Leslie , Michael Kleinhenz, Johann Coetzee, Victor Gomez Leon, Mikaela Weeder, Andrew Curtis, Andreia Machado, Santiago Hurtado

Objectives
Luteolytic drugs are critical for managing reproductive success in dairy cows. Currently, prostaglandin analogs are only approved for intramuscular injection. The utilization of a needle and syringe for the administration of any drug can result in needle-stick injuries to workers. Furthermore, needle-free injection systems have been shown to eliminate transmission of bloodborne pathogens, such as Anaplasmosis marginale, in cattle. Data on the efficacy of needle-free injection systems and their application in an estrus synchronization protocol has not been reported. The objective of the present study was to evaluate the effectiveness of utilizing a needle-free injection system [Pulse NeedleFree Systems, Lenexa, KS] for the intramuscular delivery of cloprostenol [Estrumateâ, Merck Animal Health, Madison, NJ] for the purpose of luteolysis during estrus synchronization in lactating dairy cows.
Material and methods
Lactating Holstein dairy cows (*n* = 26) were randomly assigned to treatment groups utilizing a simple randomized controlled trial study design. Treatment groups consisted of: 1) needle-free injection of 2 mL (0.5 mg) cloprostenol IM (NFREE-PG; n = 10); 2) traditional needle injection of 2 mL (0.5 mg) cloprostenol IM (NDL-PG; n = 10); 3) needle-free injection of 2 mL physiological saline IM (CNTL; n = 6). All cows were synchronized to achieve an active corpus luteum (CL) at time of treatment (60 ± 3 DIM). A CL was deemed active if the diameter was greater than 13 mm and blood flow was > 25% as determined via ovarian ultrasound. Biomarkers were evaluated at 1 h prior to treatment administration and for 92 h after treatment administration and included ovarian ultrasound measurements of corpus luteum blood flow and diameter (mm), plasma for cortisol analysis, and plasma for progesterone analysis. Data were analyzed using a mixed model with the cow as the experimental unit, utilizing commercially available statistical software.
Results
Not all results are presented. Results with significant effects are presented as least squares mean ± SE. There was no difference between the NFREE-PG (-40.7 ± 7.3 %) and NDL-PG (-39.0 ± 4.2 %) groups between 0H to 92H for percent change in CL diameter, however, the CNTL (5.8 ± 8.0 %; P<0.0001) group observed a smaller percent change in CL diameter between 0H to 92H. There was no difference between the NFREE-PG (-76.3 ± 5.2 %) and NDL-PG (-72.7 ± 5.4 %) between 0H to 92H for percent change in CL volume, however, the CNTL (3.2 ± 10.2 %; P<0.0001) observed smaller percent change in CL volume between 0H to 92H. There was no difference between the NFREE-PG (-93.1 ± 2.8 %) and NDL-PG (-97.1 ± 2.2 %) groups between 0H to 92H for percent change in CL blood flow, however, the CNTL (-0.7 ± 6.2 %; P <0.0001) observed a smaller change in CL blood flow between 0H to 92H. The NFREE-PG cows had lower cortisol levels (9.8 ± 1.2 ng/mL) compared to the NDL-PG (17.8 ± 1.2 ng/mL) and CNTL (19.2 ± 1.3 ng/mL; P = 0.04).
Conclusions
Similar regression of the CL was observed in both cloprostenol treatment groups from 20H to 92H. This indicates an effective dose of cloprostenol for luteolysis was administered via the needle-free system. For animals in the CNTL group, CL regression was not observed. Additionally, lower cortisol levels were observed in the NFREE-PG group when compared to the NDL-PG and CNTL groups. These data support the use of a needle-free injection system as a low-stress alternative to needle and syringe to deliver cloprostenol for reproductive management in lactating dairy cows. Further research is warranted to evaluate the replacement of a traditional needle injection with needle-free delivery of reproductive management drugs used in estrus synchronization protocols.

1210 - Immunoglobulin G levels of dairy-sourced calves at the point of entering the beef rearing unit, measured using a novel lateral flow device, and their association with performance during rearing.

Author: Benjamin Barber, Rachel Hayton, Alastair Hayton, Joe Dunbar

Objectives
In the UK there are an increasing number of calves that are being sourced from multiple dairies at a few weeks of age with the intention of rearing them for beef. Entering the rearing unit can be a high risk time for disease with calves that have an increased likelihood of having a failure in transfer of passive immunity (FTPI) being transported, mixed and managed differently.
Measuring immunoglobulin G (IgG) level in calves, particularly in the first week of life, is recognised as a method of evaluating FTPI. The objective of this study is to determine if IgG level in calves aged between 14 and 56 days, measured using a novel calf-side lateral flow device at the point of entering the rearing unit, is associated with future performance during rearing.
Materials and Methods
Between February 2022 and January 2024, calves that entered a single large rearing unit based in Somerset, UK, were sampled to determine their IgG level. The blood sample used was acquired via a lancet applied to the calf's nose. The lateral flow device [ImmunIGY – Bovine IgG®,Bimeda], used in conjunction with an immunochromatographic reader, gives a quantitative direct measure of IgG.
Alongside IgG, each calf had its weight, age, breed, sex and dairy herd of origin recorded on entry. Calf weights after 6 and 10 weeks of rearing, and all mortalities and medical treatments up to 10 weeks of rearing, were recorded.
Generalized Linear Models with a binomial distribution were used for the univariable and multivariable analyses assessing the odds of receiving a treatment or multiple treatments. A Gaussian distribution was used for DLWG (daily live weight gain).
Results
1735 calves enrolled onto the study had a record of medical treatments during their 10-week rearing period. 1434 had a 6-week weight and 1462 had a 10-week weight.
26% of animals had IgG levels below 10.0g/L on entry, and 12% of animals were below 7.5g/L.
Median IgG level varied between age on entry category with 14-27d, 28-41d, and 42-56d having levels 14.7, 13.6, and 12.1g/ml respectively.
Calves aged 14-27d at entry showed evidence of an IgG level below 7.5g/L being associated with a higher odds of being treated for pneumonia (OR: 2.13; 95%CI: 1.26-3.78; p-value: 0.007) and of being treated multiple times for pneumonia (OR: 2.15; 95%CI: 1.41-3.32; p-value:0.0005). Calves aged 14-27d also showed evidence of having a higher odds of receiving a treatment when IgG levels were below thresholds 10.0g/L (OR: 1.72; 95%CI: 1.16-2.60; p-value: 0.008) and 12.5g/L (OR: 1.61; 95%CI: 1.14-2.30; p-value: 0.008).
Calves aged 28-41d at entry showed evidence of an IgG level below 7.5g/L being associated with a higher odds of being treated for pneumonia (OR: 2.06; 95%CI: 1.19-3.75; p-value: 0.01) and of being treated multiple times for pneumonia (OR: 2.16; 95%CI: 1.34-3.53; p-value:0.002). Calves aged 28-41d also showed evidence of having a higher odds of receiving a treatment when IgG levels were below 10.0g/L (OR: 1.50; 95%CI: 1.03-2.21; p-value: 0.04).
There was some evidence that calves aged 14-27d at entry which were sourced from a 'lower quartile' dairy (where ranking was based on the proportion of calves above 10g/L) had a higher odds of being treated (OR: 1.51; 95%CI: 0.94-2.51; p-value: 0.09) and of receiving multiple treatments (OR: 2.04; 95%CI: 1.35-3.11; p-value:0.0007) regardless of their own individual IgG level.
There was also some evidence of calves aged 14-27d entry having reduced 6-week DLWG (β:-0.044; 95%CI: (-)0.089-0.002; p-value: 0.05) when arriving with IgG levels below 7.5g/L.

There was no evidence of an association between performance or health parameters, and IgG level within the age on entry category 42-56 days.

Conclusions

Evidence of associations between low IgG level on entry to the rearing unit and reduction in health parameters, for calves between 14 and 42 days old, is encouraging for potential early identification of sub-optimal calves.

This system of obtaining blood has not been used previously in cattle and would allow unskilled operators to sample.

1575 - Clinical and cytological cervix findings and occurrence of postpartum uterine diseases in grazing dairy cows from high-altitude tropical herds

Author: Dario Antonio Vallejo Timarán, Patricia Betancourth, Sebastian Amaya, Isabela Perez

Objectives
The objective of this study was to quantify the incidence risk of clinical and cytological cervicitis in early postpartum dairy cows from high-altitude tropical dairy herds in a pasture-based systems and, to evaluate the association with the occurrence of metritis, clinical endometritis, cytological endometritis and the occurrence of anovulation.
Material and methods
A total of 71 Holstein cows from three commercial dairy herds in north Antioquia, Colombia, were enrolled in this prospective observational longitudinal cohort study. The sample size was calculated at convenience based on the total of deliveries occurred in the herds included in the study in a period of 6 months. Each cow was examined at 30, 45, and 60 days postpartum. Clinical examinations include vaginoscopy, transrectal palpation, uterine/ovarian ultrasound, endometrial cytology, and cervical cytology. Cows were included in the study based on the following criteria: dairy breed (e.g. Holstein), non-interventional calving by caesarean section, no clinical signs of systemic disease (e.g. depressed attitude, anorexia, fever, downer cow) that required the use of antibiotics and anti-inflammatories. Information about breed, parity, occurrence of retained placenta, body condition score was collected. Additional data on grazing, supplementation and transition period feeding were also collected. The diagnosis of cervicitis was made from day 30 postpartum through Vaginoscopy, the cervix was classified as: Grade 0 (Normal cervix, without prolapse of the second cervical ring and without congestion); Grade 1 (Normal, redness of the cervix, without prolapse of the second cervical ring); Grade 2 (Clinical cervicitis, redness (congestion) of the cervix with prolapse of the second cervical ring). Through endometrial cytology, cytological cervicitis was diagnosed with a percentage of neutrophils >5%. Transrectal palpation and uterine/ovarian ultrasound were also performed, for the diagnosis of other uterine conditions (clinical/cytological endometritis). The variables included in the study were: calving variables (type of calving, body condition score, blood calcium levels, parity); clinical variables (uterine involution, ovarian status, postpartum uterine diseases, clinical cervicitis, cytological cervicitis). The data were analyzed using descriptive statistics, incidence risk was calculated by the difference between the number of animals with the condition and the total of evaluated animals. The association between cervicitis and postpartum uterine diseases was evaluated through non-parametric test. Association between calving variables and cervicitis was evaluated through logistic regression models, same analysis was performed to found association between cervicitis and fertility variables. For the selection of the models and the control of biases and confounders, a Directed Acyclic Graph was constructed.
Results
Dystocia and retained placenta occurred in 17% and 14% respectively. The percentage of subclinical hypocalcemia was 56%. The incidence risk of Metritis, clinical endometritis and cytological endometritis was 37%, 32% and 34% respectively. Anovulation was diagnosed in the 59% of the cows at 60 days postpartum. The 46% and 32% of cows were diagnosed with clinical and cytological cervicitis respectively at 60 days postpartum. About 64.2% of cows with clinical endometritis at 60 days postpartum also had clinical cervicitis ($P \leq 0.05$) and 25% of cows with cytological vaginitis had clinical cervicitis. Significant association ($p<0.05$) was found between the diagnosis of grade 2 cervix on day 45 postpartum with the subsequent development of purulent vaginal discharge on day 60 postpartum. The 76% of the cows diagnosed with anovulation had clinical endometritis ($p<0.05$). No association was found between subclinical

hypocalcemia and the occurrence of clinical cervicitis. No direct relationship was found between the occurrence of clinical cervicitis and anovulation.

Conclusions

In grazing dairy cows from high tropics herds, clinical cervicitis was associated with the occurrence of clinical endometritis which affected the fertility of the cows evaluated. Furter research it is recommended to evaluate whether visual vaginal examination at 30 and 45 days postpartum can predict the subsequent occurrence of clinical endometritis. In the same way, it is recommended to evaluate whether the therapeutic management of clinical cervicitis can reduce the subsequent occurrence of clinical endometritis.

1572 - The effect of heat stress on periparturient diseases in dairy cows

Author: Muhittin Tekin, Mauricio Javier Giuliodori, Christian Guse, Michael Iwersen, Marc Drillich, Karen Wagener

Objectives
In the dairy industry, heat stress (HS) has become one of the central concerns due to global warming and its significant impact on the health and productivity of dairy cows. Therefore, the aim of this study was to assess the effect of HS on the occurrence of periparturient diseases.
Material and methods
This study was conducted on a commercial dairy farm located in Slovakia, and farm visits that based on herd management of the farm were always on the same day of each week. A total of 1,818 cows with two to eight lactations were included in the study on the day of calving. After calving, animals underwent diagnosis for subclinical hypocalcaemia (SCH), puerperal metritis (PM) and subclinical ketosis (SK) on day five postpartum (pp), as well as clinical/subclinical endometritis (CE/SE) on day 28 pp. After a voluntary waiting period of 50 days, cows detected in oestrus were bred by artificial insemination (AI), and cows not detected in oestrus by day 70 pp were subjected to an OvSynch protocol with timed AI. Pregnancy diagnosis was conducted at 42 and 93 days post-AI by ultrasound. The body condition scores (BCS) were assessed and BCS-loss between calving and day 28 pp was calculated. Ambient temperature and relative humidity in the barn were recorded by digital loggers at 30-minute intervals (TGP-4500, Gemini Data Loggers Ltd., the UK). To explore the impact of HS a temperature humidity index (THI) was calculated with the comfort threshold defined as THI ≥ 68. The level of HS was determined by calculating the accumulated area between the THI threshold and the amplitude approximated by the Riemann sum.
Results
In this study, it was found that the first service conception rate was 41%. Cows that experienced HS during the first five days pp were more likely to have SK than cows without HS (Odds Ratio (OR): 1.82; Confidence interval (CI): 1.52–2.18; $P < 0.01$). Cows under HS during the first five days pp had an increased risk for metritis compared with cows in the comfort zone (OR: 1.39; CI: 1.05–1.84; $P < 0.01$). Moreover, cows diagnosed with SCH had a greater risk for metritis (OR: 1.30; CI: 0.76–2.20; $P < 0.01$). HS during the first 28 days pp did not affect occurrence of CE and SE ($P > 0.05$). Cows exposed to HS during the first 28 days pp had greater BCS-loss than those without HS (OR: 0.98; CI: 0.94–0.99; $P < 0.05$).
Conclusions
In summary, these findings illustrate that HS under Central European conditions represents a risk factor for an increased susceptibility to different periparturient diseases. Our findings should be confirmed with data from a greater number of farms.

1523 - Endometrial macrophage populations in the establishment and persistence of subclinical endometritis of postpartum dairy cows

Author: Catarina Anastácio , Gonçalo Pereira, Elisabete Silva, Ricardo Bexiga, Luís Capela, Patrice Humblot, Luís Lopes da Costa

Objectives

Subclinical endometritis (SCE), often progressing to a persistent status leading to postpartum dairy cow subfertility/infertility, is a worldwide concern of the dairy industry. Diagnosis requires evaluation of inflammation in endometrial cytological or biopsy samples. While the characterization and role of polymorphonuclear neutrophils (PMN) populations are well established in these samples, the characterization and role of endometrial macrophages remain unclear.

The aim of this study was to characterize the endometrial macrophage population in postpartum dairy cows, and its relationship with the establishment and persistence of SCE.

Material and methods

Cows without puerperal clinical disease (n=173) from a high-yielding Holstein dairy herd were enrolled in the study. Sampling considered uterine cytology at 21 and 42 days postpartum (DPP), a uterine flush of the previously pregnant uterine horn at 21 DPP, and an endometrial biopsy at 42 DPP. According to uterine cytology (Diff-Quick stain), cows were retrospectively allocated to groups Healthy (n=86; PMN<18% at 21 DPP and PMN <5% at 42 DPP), Recovered SCE (SCER; n=56; PMN≥18% at 21 DPP and PMN<5% at 42 DPP) and Persistent SCE (SCEP; n=31; PMN≥18% at 21 DPP and PMN≥5% at 42 DPP).

Macrophage pro-inflammatory M1 (CD86+), anti-inflammatory M2 (CD163+) and total (CD68+) populations were evaluated by immunocytochemistry (ICC) in uterine cytology samples in a set of animals of each group (Healthy, n=7; SCER, n=9; SCEP, n=4). Protein abundance of M1 (CD86) and M2 (CD163) macrophages was analyzed by Western blot with densitometry in the cellular pellet of uterine flush samples (Healthy, n=19; SCER, n=11; SCEP, n=4). Total (CD68+) and M2 (CD163+) macrophage populations were evaluated by immunohistochemistry (IHC) in 42 DPP uterine biopsy samples (Healthy, n=20; SCER, n=18; SCEP, n=11). The proportion of total, M1 and M2 macrophages, and the M1:M2 ratio was compared between groups by one-way ANOVA. Spearman correlations were calculated between the proportion of uterine cytology Diff-Quick stained and ICC labelled total (CD68+) macrophages in samples of 21 and 42 DPP, and the ICC labelled macrophage phenotype M1 (CD86+) and M2 (CD163+) and their protein abundance detected by Western blot.

Results

The proportion (%) of uterine cytology Diff-Quick stained macrophages at 21 DPP was higher in SCER than in Healthy and SCEP cows (5.5±0.5 vs. 2.5±0.4 and 1.4±0.7; P<0.01). At 42 DPP, this proportion was higher in SCEP than in Healthy and SCER cows (1.6±0.3 vs. 0.8±0.1 and 0.8±0.2; P<0.05).

At 21 DPP, in ICC labelled uterine cytology, the proportion (%) of anti-inflammatory M2 macrophages was higher in SCER than in Healthy and SCEP cows (2.3±0.6 vs. 0.3±0.4 and 0.1±0.8; P<0.05), and their protein abundance in the uterine cellular pellet was also higher in SCER than in Healthy and SCEP cows (4.1±0.5 ADU vs. 1.7±0.6 ADU and 0.8±0.7 ADU; P<0.05). In ICC labelled uterine cytology, the M1:M2 ratio was higher in SCEP than in Healthy and SCER cows (4.9±1.3 vs. 0.5±0.9 and 0.5±0.9; P<0.01).

The uterine cytology Diff-Quick stained macrophage proportion strongly correlated with ICC labelled total macrophages (CD68+) at 21 DPP (r=0.930; P<0.0001) and 42 DPP (r=0.925; P<0.0001). At 21 DPP, the uterine cytology ICC labelled M2 (CD163+) macrophage proportion strongly correlated with total (CD68+) macrophage (r=0.843, P<0.0001) and Diff-Quick stained

macrophage (r=0.884, P<0.0001) proportions. The ICC labelled M1 (CD86+) and total (CD68+) macrophage proportions also correlated (r=0.714, P<0.0001), but only a moderate correlation was found between ICC labelled M1 (CD86+) and Diff-Quick stained macrophage proportions (r=0.477; P<0.05). M2 (CD163) protein abundance had a moderate correlation (r=0.422; P <0.05) with uterine cytology Diff-Quick stained macrophage proportion.

Despite of the lower uterine cytology macrophage proportion at 42 DPP, the endometrial biopsy sample total macrophage counts (2.4±0.3 cells/field vs. 1.4±0.2 cells/field; P<0.05) and M2 macrophages counts (1.7±0.2 cells/field vs. 1.0±0.2 cells/field; P<0.05) were higher in SCEP than in SCER cows.

Conclusions

Evaluation of macrophage proportions in uterine cytology at 21 DPP allowed a prognosis for the recovery or persistence of SCE. Within the macrophage population, the M2 phenotype is linked to the resolution of endometrial inflammation, whereas a high M1:M2 ratio is linked to the persistence of the inflammation. At 42 DPP, the macrophage content in endometrial cytology samples is low, but the endometrial biopsy showed their presence in deeper layers of the endometrium, where their number is higher in cows with persistent inflammation, suggesting a delayed response.

1501 - Association of lochia cytokines and serum amyloid A during the early postpartum period with subsequent reproductive performance in eutocia and dystocia beef cows

Author: Takeshi Osawa, Minami Yatabe, Shiori Kohigashi, Kazuhiro Kanemaru, Go Kitahara

Objectives

Healthy parturition and uterine involution during the puerperium are necessary to maintain normal fertility in cows. We have reported that pre-partum cervical ripening is accompanied by a physiological inflammatory response [Yamanokuchi *et al*., 2022]. A transient inflammatory response in the reproductive tract is also known to occur in the early postpartum period [Sheldon *et al*., 2019]. On the other hand, reproductive performance is reduced in dystocia animals having severe injury of the reproductive tract. However, the dynamics of inflammation in the reproductive tract in dystocia cows remain unclear. Therefore, this study was conducted to elucidate the association of calving assistance and the inflammatory response in the early postpartum period with subsequent reproductive performance in eutocia and dystocia beef cows.

Material and methods

A total of 147 Japanese Black cows (age: 5.7 ± 2.3 years, parity: 4.0 ± 2.0), in a farm and calved between May 2022 and April 2023, were tested. At 7 ± 3 days (W1) and 14 ± 3 days (W2) postpartum, lochia and blood were collected. The lochia samples were homogenized and cytokine [interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-10, TNF-α] concentrations were determined in a multiplex assay instrument. Serum was separated from blood samples and serum amyloid A (SAA) concentrations were measured by latex agglutination turbidimetric immunoassay. The cows were divided into the eutocia group (n = 88), and the dystocia group (n = 59), which required calving assistance, to compare cytokines and SAA levels and their relationship to subsequent reproductive performance.

Results

Lochia concentrations of IL-1α, IL-1β and IL-6 at W1, and IL-1α, IL-1β and TNF-α at W2 in the dystocia group were higher ($P < 0.05$) than in the eutocia group. SAA concentrations in the dystocia group were higher compared with the eutocia group in W1 ($P = 0.06$) and in W2 ($P < 0.05$). When comparing reproductive performance between the top and bottom third of cytokine concentrations in the dystocia group, days open were fewer ($P < 0.05$) in the bottom third of IL-10 concentrations at W1, days at first artificial insemination (AI) were shorter ($P < 0.05$) in the bottom third of IL-1α and IL-8 concentrations at W2, and the top third of IL-6 concentrations at W2 had a lower ($P < 0.05$) number of AI required for conception.

Conclusions

The dystocia group experienced a stronger inflammatory response from the first to the second week postpartum. In particular, sufficient inflammation occurred by the first week postpartum, suggesting that the convergence of inflammation by the second week postpartum was a factor that did not reduce the fertility of the dystocia cows. This study has revealed some of the dynamics of inflammation in the reproductive tract that leads to normal reproductive performance in dystocia cows. Clarification of dynamics of inflammatory and anti-inflammatory cytokine and SAA in the early postpartum period, which may reduce reproductive performance, could be applied to the early detection and early treatment of low fertility cows.

1644 - The effect of heat stress on reproductive performance of dairy cows

Author: Muhittin Tekin, Mauricio Javier Giuliodori, Christian Guse, Michael Iwersen, Marc Drillich, Karen Wagener

Objectives
Over the last decades, there has been a dramatic increase in ambient temperature caused by global warming. Thus, heat stress (HS) is becoming an increasingly important issue in the dairy industry as it has detrimental effects on the health and productivity of cows. Although research has been conducted on the effect of HS on fertility in general, less is known about the-related effects of HS exposure around artificial insemination (AI). Thus, this study aimed to evaluate the impacts of accumulated HS at various intervals around AI on the subsequent reproductive performance of dairy cows.
Material and methods
The present study was carried out on a commercial dairy farm located in Slovakia, and farm visits consistently scheduled to align with herd management of the farm on a weekly basis. A total of 1,818 animals with a mean lactation number of 3.04 were enrolled in the study. Body condition scores (BCS) were assessed at calving and day 28 postpartum (pp) to calculate the BCS loss. After a voluntary waiting period of 50 days, cows detected in oestrus were artificially inseminated. Cows not showing oestrus signs were subjected to an OvSnych protocol with timed AI. Pregnancy checks were performed at 42 and 93 days after AI. To measure climatic conditions, ambient temperature, and relative humidity were recorded every 30 minutes using calibrated digital loggers (TGP-4500, Gemini Data Loggers Ltd., the UK) placed in the barn. To assess the HS exposure, the temperature humidity index (THI) was determined, and the comfort zone of the cows was set at THI ≤ 68. For the approximation of accumulated HS levels, the area under the curve between the THI threshold and the amplitude was calculated using Reimann sums. Subsequently, accumulated THI values above the threshold were added up.
Results
Conception rate to the first service was 41% in the present study. The association of HS exposure around the time of AI (weeks 1, 2, 3, 4, 5, 6 before and after AI) with different reproductive parameters was evaluated. HS did not affect the hazard of insemination (Hazard Ratio (HR): 0.97 ; Confidence interval (CI): 0.87–1.08; $P > 0.05$), but, conversely, HS affected the hazard of pregnancy (HR: 0.825; CI: 0.72–0.94, $P < 0.05$). The odds for pregnancy for all services (ranging from 1 to 6 AI) were lower in cows under HS during the first 42 days after AI compared with cows under HS during 42 days before AI (Odds Ratio: 0.89; CI: 0.81–0.99; $P < 0.05$). Further analysis showed that cows were less likely to become pregnant when they experienced HS one week before AI than cows who experienced HS six weeks before and after AI ($P < 0.05$). There was no significant effect of HS on the first service conception rate ($P > 0.05$).
Conclusions
Taken together, these findings indicate that HS did not affect the hazard of insemination, but it negatively affected the hazard of pregnancy. Also, HS did not affect the odds for pregnancy at first insemination, but conversely, HS around insemination (6 weeks before and after AI) was negatively associated with pregnancy, especially in the week before insemination. However, further research is needed to understand the link between HS, health, and fertility.

1162 - Anti-Müllerian Hormone (AMH) concentrations and weaning weight in beef heifers born to cows subjected to mid-pregnancy weaning stress

Author: Brian Birkenhagen, Sanjaya Mijar, Karin Orsel, Juan Hernandez-Medrano

Objectives
Maternal stress during pregnancy alters fetal organ and tissue development with long-term health, welfare, and reproductive implications in cattle (Perry *et al*., 2019; Reynolds *et al*., 2022). Weaning stress is a common maternal stressor in beef cattle (Ungerfeld *et al*., 2011) occurring around mid-pregnancy, coinciding with the establishment of the ovarian follicular reserve (OR). Anti-Müllerian hormone (AMH) is considered a marker of OR and linked to adult fertility in cattle (Alward *et al*., 2019). This study explored the fetal effects of maternal weaning stress on calf's AMH plasma concentrations and weight at weaning (5-mo of age).
Material and methods
Heifer calves (n=26) born to adult cows subjected to 3 different weaning strategies during mid-pregnancy (nose-flap, NF, n=12; fence-line, F, n=8; or abrupt, A, n=6), as part of a previous experiment (Mijar *et al*. 2023), were used in this study. Fetal age at maternal weaning was calculated by subtracting the average gestation length (Angus = 283 d) from the calving date for each calf. Calves were blood sampled and weighed at weaning (mean age = 165.9±2.9 d), as part of the routine management. Plasma AMH concentrations were estimated using a bovine-validated ELISA test (Ansh Labs Bovine AMH ELISA kit; intra- and inter-assay CV% = 3.1% and 7.8%, respectively). Non-normally distributed data was transformed (Box-Cox) and analyzed using ANCOVA with fetal age as covariate (Minitab v21, 2023).
Results
Maternal weaning occurred earlier in abruptly weaned (A, 105.3±5.6 d) compared to F and NF groups (140.1±3.6 d and 131.1±4.9 d, respectively; P<0.001). Results from the associated cow weaning experiment (Mijar *et al*., unpublished) showed that cows in NF group had higher cortisol in the days following weaning compared to A and F cows. AMH concentrations at weaning (~5-mo of age) tended to be lower in NF (798.3±140.7 ng/ml) compared to A and F heifer calves (1276.3±179.2 ng/ml, and 1315.8±241.1 ng/ml; P=0.079). Moreover, calves born to cows in the A and NF groups were heavier at weaning (611.6±23.2 kg, and 602.9±17.3 kg, respectively) than those from the F group (555.7±16.8 kg; P=0.042).
Conclusions
Fetal stress around mid-gestation, as a result of the cow experiencing stress at weaning, tended to decrease AMH concentrations and increase body weight in 5-mo old heifer calves, which may indicate an impact on offspring's reproductive performance (decreased OR and fertility) and growth (weaning weights). This study shows the potential long-lasting effects of maternal stress on offspring productive and reproductive performance.

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Sheep and goats

1025 - Milk and Tissue Residue Depletion of Two Dry-Cow Intramammary Antimicrobials in Dairy Goats

Author: Michelle Plotzker Buckley, Kristen Hayman, Dwayne Schrunk, Laura Burns, Patrick Gorden

Objectives

The goal of this study was to determine milk and tissue residue depletion times of cephapirin (CEPH) and cloxacillin benzathine (CLOX) after intramammary administration to dairy goats at dry-off.

Material and methods

Milk

To evaluate milk residue depletion, 22 does had each half treated with 300 mg CEPH [ToMORROW, Boehringer Ingelheim Vetmedica, Duluth, GA] after their final milking before dry-off, while an additional 21 does were treated with 500 mg CLOX [Orbenin DC, Merck & Co., Rahway, NJ] in each half. Starting at the first milking after kidding, quadruplicate composite foremilk samples were collected from each doe for evaluation of antimicrobial residues. Two samples were utilized immediately after milking for goat-side evaluation of drug residue while two were stored at -20°F for later analysis by LC-MS-MS. Does treated with cephapirin were screened using the Charm SLBL assay [Charm Sciences Inc., Lawrence, MA] which is approved for detection of this drug in goat milk. Does treated with cloxacillin were evaluated using the Charm SL3 Beta-lactam assay [Charm Sciences Inc., Lawrence, MA], which was assessed for use in goat milk using samples spiked with known concentrations of drug by the ISU research group before initiation of this project. Does were sampled at each milking until they tested negative on the goat-side tests for two consecutive milkings via the goat-side assay.

Tissue

Forty-two lactating goats with two functional halves were enrolled and randomly assigned to treatment groups, 21 does received one intramammary dose of CEPH into each teat after their final milking before dry-off. Twenty-one additional does received one dose of CLOX in each half of the udder at their final milking before dry-off. One doe died of undetermined causes during the holding period. Four does from each treatment group were sacrificed at 21, 28, 35, 42, and 49 days post-treatment and 200 g of muscle, liver, and kidney tissue harvested from each animal from analysis of antimicrobial residues using LC-MS-MS.

A second study was completed after tissue processing in order to more fully elucidate the pharmacokinetic properties of cloxacillin in tissues. During this second phase, six additional does were treated with 500 mg CLOX in each half immediately following their final milking. Three of these does were sacrificed on day 3 post-treatment while the remaining three does were sacrificed 7 days post-treatment. Samples were collected and processed in the same manner as in the original sample collection.

Results

Milk

On LC-MS-MS, 18 of 22 cephapirin-treated does (81.8%) tested below the level of quantification (5 ppb) at the first milking. Three does had quantifiable residues at the first milking, with no residues detected in any animal after 60 hours. CLOX-treated does had a less uniform response with 10 out of 21 does (48%) having no quantifiable residue at first milking, 3 does (14%) had no

detectable residue by the second milking, and the remaining 8 does (38%) had residues for 36 hours or more with a mean of 84 hours required to clear detectable residues. One doe was unenrolled from the study at 144 hours post-kidding for prolonged residue production with a final reading of 57.3 ppb on LC-MS-MS.

Tissue

Cephapirin was not identified in any tissue at any point during the sampling period. Cloxacillin was not found in muscle tissue at any point during the sampling period. Peak concentrations of 0.0025 ppm and 0.0026 ppm for kidney and liver, respectively, were found at 3 days post-treatment. Cloxacillin was identified in all liver and kidney samples within the first 7 days post-treatment, 100% (4/4) of kidney samples at 21 days post-treatment, 50% (2/4) of liver and 25% (1/4) of kidney samples from 28 days post-treatment, and 50% (2/4) of livers and kidneys at 35 days post-treatment with no further residues detected after this time point. At no point was cloxacillin concentration greater than the current tolerance of 0.01 ppm that has been established for bovine tissues.

Conclusions

This study demonstrated prolonged detection of CLOX in milk and tissue as compared with CEPH. These findings align with the pharmacokinetic data in dairy goats also produced by this group indicating that CEPH has a markedly shorter plasma persistence time than CLOX. Our working hypothesis is that this is due to a lower solubility of the cloxacillin benzathine complex in the udder as compared to the cephapirin benzathine complex.

1019 - Is crossbreeding with Boer the answer to improve live weight and growth of Nubian kids in flocks of Colima, Mexico?

Author: Julio Alejandro Rangel Sagrero, Rafael Julio Macedo Barragán, José Luis Zepeda Batista, Victalina Arredondo Ruiz, Mauricio Valencia Posadas, Carlos Alberto García Munguía

Objectives
The objective of this study was to evaluate live weight and growth of Boer and Nubian kids and their crosses in Colima, Mexico.
Material and methods
Records of 452 kids raised on five farms including information on breed, sex, litter size, and weights at birth, weaning (90 days), 180 and 365 days were used. To evaluate the fixed effects of breed, sex and litter size on live weight and pre-weaning and post-weaning growth rate, the information was analyzed using an analysis of variance with a completely randomized design and a factorial arrangement. In addition, the growth curves of the kids were estimated using the non-linear Gompertz regression model.
Results
Boer kids showed the greatest ($P < 0.05$) birth weight and together with 7/8 kids the greatest ($P < 0.05$) weaning weight and the pre-weaning growth rate. Weight at 180 days were smaller ($P < 0.05$) in F_1 kids while weight at year and post-weaning growth rate were greatest ($P < 0.05$) in Boer kids. Kid sex did not affect ($P > 0.05$) birth weight as well as weaning weight and pre-weaning growth rate was greater ($P < 0.05$) in female kids. Weight at 180 and 365 days and post-weaning growth rate was greater ($P < 0.05$) in male kids. Single and twin kids had the greatest ($P < 0.05$) birth weight and single kids the greatest ($P < 0.05$) weaning weight and pre-weaning growth rate. Weight at 180 days was similar ($P > 0.05$) in all kids, but weight at 365 days and post-weaning growth rate were greater ($P < 0.05$) in twins and triplets. According to Gompertz model Boer, male and twin kids showed the greatest value for the parameter A, which represents the asymptotic weight, and the lowest value for the parameter k, which represents the maturation rate. Also, 3/4 Boer, female and single kids showed the lowest value for parameter A and the highest maturation rate. Growth curves showed that growing of all the genotypes was similar during the first 45 days and from weaning (90 days) Boer kids presented a higher growth rate up to 365 days. Furthermore, growth of male and female as well as single, twin and triplet kids was very uniform during the first 90 and 135 days respectively, and after that age, male, twin and triplet kids showed a higher growth rate and consequently, a greater weight at year.
Conclusions
It is concluded that terminal crossbreeding (F_1) with Boer did not demonstrate to be a viable option to increase live weight and growth of Nubian kids in Colima, Mexico whose productive performance improved as was absorbed by Boer breed.

1017 - Mannheimia haemolytica vaccination of pregnant goats to enhance the transfer of passive immunity against pneumonic pateurellosis.

Author: Pleun Penterman, Stef van Roessel, Henk Kuijk, Geert Vertenten, Bart Sustronck

Objectives

Pneumonic pasteurellosis is a major problem in the dairy goat industry in Europe and is comparable to the pneumonic pasteurellosis encountered in sheep. One of the principal pathogens involved in this multifactorial disease complex is *Mannheimia haemolytica*. Pneumonic pasteurellosis in goat kids very often occurs at young age. Therefore, transfer of passive immunity is extremely important to protect goat kids against this respiratory disease. It is known that vaccination of pregnant sheep against *M. haemolytica*, using Ovipast® Plus (MSD Animal Health, The Netherlands) induces significantly higher *M. haemolytica* serum antibody titers in their lambs compared to lambs originating from unvaccinated ewes. However, it is unknown whether off-label use of Ovipast® Plus in pregnant goats has a similar passive immunity enhancing effect. So, the main objective of the study is to demonstrate that the use of Ovipast® Plus in pregnant goats improves the passive transfer of immunity against *M. haemolytica* towards new-born goat kids.

Material and methods

The experimental design consisted of a controlled longitudinal field trial on a Dutch dairy goat farm. Two groups of pregnant goats and their offspring were followed. The first group consisted of 20 pregnant goats that were vaccinated twice with Ovipast® Plus. A second group of 20 pregnant goats served as negative control group. The kids received colostrum only from their own mothers according to the routine colostrum management applied in the farm. During the follow-up period, serum antibody titers against *M. haemolytica* were determined in the goats and kids at regular intervals. All statistical analyses were performed using R software (R Core Team, 2017). Significance was set at $p<0.05$.

Results

The *M. haemolytica* serum antibody titers in the two groups of pregnant goats were comparable prior to the vaccination. At the time of kidding, 4 weeks after the second vaccination, the *M. haemolytica* serum antibody titer was significantly higher in the vaccinated goats (mean±sd: 10.01±1.31) compared to the non-vaccinated controls (9.14±1.21).

Goat kids that received colostrum from vaccinated goats had numerically higher serum *M. haemolytica* antibody titers (7.03±1.04) at the age of 2 to 7 days compared to kids that received colostrum from non-vaccinated goats (6.15±1.23) ($p=0.10$). At weaning the two groups of goat kids had comparable *M. haemolytica* serum antibody titers.

Conclusions

The vaccination of pregnant goats with Ovipast® Plus resulted in an improved passive immunity against *M. haemolytica* in their offspring as evaluated by the level of serum antibodies. However, this induced passive immunity in goat kids doesn't seem to last very long . For the practitioner this information could be useful when applying the vaccine according to the legal cascade regulations that are in place.

1286 - Diagnostic Methods for Assessing Transfer of Passive Immunity in lambs

Author: Ana Laura Martins, Raquel Fraga e Silva Raimondo

Objectives

Ruminants are born with deficient concentrations of gamma globulins, and their immune system does not yet have total response capacity. This characteristic occurs due to the synepitheliochorial interhemal barrier of the placenta that does not allow the transfer of immunity. The absorption of immunoglobulins, especially class G (IgG), present in the mother's colostrum guarantees protection in the early period of life. There is a large amount of information on passive immunity transfer (PIT) in cattle, while research is scarce in sheep. Therefore, this research aimed to evaluate different diagnostic methods for the transfer of passive immunity in lambs

Material and methods

13 mixed-breed lambs were used, three of which were twin gestations, born from eutocic birth of 10 pluriparous ewes. Blood samples from the lambs were collected by jugular venipuncture using the vacuum system after natural colostrum feeding. The blood was centrifuged at 1000g for 15 minutes to obtain blood serum. To evaluate PIT, the serum was evaluated using the Brix methodology on a digital and analog device and using the immunocyte method, and, as a gold standard, total serum protein was evaluated by refractometry. The immunocrit is an indirect test that uses ammonium sulfate solution [(NH4)2SO4] to precipitate serum immunoglobulins. The means and standard deviation were calculated to evaluate the results. Total protein means by refractometry were grouped in twin and single lambs and were compared using the t-test method. To evaluate the three TIP diagnostic methods, the results of all lambs were subjected to Pearson correlation analysis, considering the serum total protein value as the gold standard. Correlation coefficients (r) were considered weak when r = 0.1 to 0.3, moderate when r = 0.4 to 0.6, and strong when r = 0.7 to 1.0. A significance level of 5% was considered.

Results

The mean serum protein assessed by refractometry was 7.567 ± 0.2591 g/dL in twin lambs and 8.471 ± 0.3834 in single lambs, and there was no difference between the groups. For ruminants, serum protein values above 5.0g/dL are recommended as a cutoff point to consider success in PIT. In the present study, all lambs had an adequate transfer of passive immunity. The average results and standard deviation obtained for the different PIT assessment methods in the total set of lambs were total serum protein 8.05 ± 0.95 g/dL; analog brix 10.32 ± 1.14%; digital brix 10.34 ± 1.10% and immunocrit 12.50 ± 3.20%. Serum total protein had a high correlation with analog brix (r =0.9795; P = 0.0001), digital brix (r = 0.9870; P = 0.0001) and immunocrit analysis (r =0 .8945; P = 0.0001).

Conclusions

The diagnostic methods evaluated: analog brix, digital brix and immunocrit showed a high correlation with serum protein obtained by refractometry and are tools that can be used routinely in the field to diagnose TIP in lambs, optimizing production and reducing mortality rates.

1664 - Small ruminant lentiviruses on different production systems in Portugal

Author: João Jacob-Ferreira, Ana Cláudia Coelho, Ana Grau Vila, Delia Lacasta, Ramiro Valentim, Hélder Quintas

Objectives
Small ruminant lentiviruses (SRLV) are a group that infect and transmit among ovine and caprine species. Diagnosis is established through laboratorial methods. The aim of this work was to study the seroprevalence of SRLV in Portugal on different production systems and also risk factors associated to this infection.
Material and methods
The seroprevalence study of SRLV was done in Portuguese flocks of ovine and caprine species. Flocks were randomly selected, and producers invited to enrol the study. According to the total of the flock population a random group of 14 to 19 blood sample was drawn from different animals. To each sample was done the indirect ELISA commercial test (ID Screen® MVV/CAEV Indirect) with the objective to detect infection. Each flock was considered to be positive if at least one animal was positive.
Results
In this study we collected a total of 200 flocks, of which 133 of ovine, 51 of caprine and 16 mixed. We verified that 160 (80%) flocks were positive to SRLV, of which 104 (78.2%) of ovine, 41 (80.4%) of caprine and 15 (93.8%) mixed.
Of 3497 individual blood samples, 1398 (40%) were positive to SRLV, with 894 (34.7%) of ovine and 504 (54.9%) of caprine positive species. Of risk factor analysis we verified a statistical association ($p<0.001$) to SRLV infection in animals older than 2 years old ((OR=2.26 – IC95%: 1.87-2.72); herds for dairy production (OR=1.76 – IC95%: 1.44-2.08); intensive herds (OR=2.19 – IC95%: 1.64-2.92), buying replacement lambs (OR=1.39 – IC95%: 1.11-1.74). We did not verify any statistical association ($p>0.05$) about animals' sex, separate unhealthy animals and about the different methods of milking.
Conclusions
We observed a high seroprevalence of SRLV in flocks of small ruminants in Portugal. Analysis of risk factors that were associated to SRLV infection contributed to a better understanding and deep knowledge about this disease. We under light the relevance of serological tests execution in early tracking of diseases in small ruminant flocks. Having epidemiological questionnaires done frequently supports the possible risk factors analysis. This knowledge empowers the effective preventable measures to be applied earlier. It should be promoted and implemented biosafety measures in order to diminish viral transmission and so diminish the prevalence of this disease. This way, we understand that governmental authorities should promote and audit control and eradication programs to eliminate this disease in ovine flocks in Portugal.

1761 - Genetic characterization and variability of Pelibuey, Katahdin, Black Belly, Saint Croix, Suffolk, Hampshire and Dorset sheep in Mexico using microsatellites

Author: Ye Eun Kim, ANTONIO ROLDÁN ROLDÁN, Javier Valencia, José Manuel Berruecos, Cecilio Ubaldo Aguilar

Objectives
Pelibuey is one of the most adapted Mexican sheep breeds to diverse geographic and productive conditions of the country, and it is going through unaware crosses with other breeds such as Katahdin, being threatened to lose its own genetic identity. For this reason, this study searches for DNA microsatellites that distinguish Pelibuey from other Mexican hair and wool breeds, describing their genetic association and distances, to be used to develop different genetic conservation and improvement strategies.
Material and methods
For this, 15 unrelated animals per breed from seven different sheep breeds across Mexico (Pelibuey, Katahdin, Black Belly, Saint Croix, Suffolk, Hampshire and Dorset) were included in this study. The DNA was extracted using modified Bailes protocol by Roldán (2015), and 9 DNA microsatellite *loci,* successfully tested in Pelibuey (Aguilar *et al*., 2021), following FAO´s recommendation were used,
Results
amplifying 192 alleles in total. Only 32 alleles were private for specific individuals with low frequency, and two alleles obtained with ILSTS5 were exclusive for Katahdin. The observed heterozygosity (Ho) from the populations ranged from 0.696 (Pelibuey) to 0.859 (Dorset), with an average of 0.785. The Shannon´s index was calculated to be from 2.119 with Pelibuey to 2.309 with Saint Croix. Also the inbreeding coefficient (Fis) was calculated, with Dorset being the lowest with 0.024 and Pelibuey being the highest with 0.195. All these values indicate that Pelibuey is characterized to have the lowest genetic diversity and to be the most inbred. The correspondence factor analysis clustered Pelibuey with Saint Croix, while Suffolk was grouped with Hampshire, both cases sharing a common geographic origin.
Conclusions
Locus ILSTS5 was also identified for amplifying two private alleles for Katahdin, which allow differentiating Katahdin from any other breed included in this study. Considering it to be the most crossed breed with Pelibuey and other hair breeds, this information can set the foundation to design further genetic tools to distinguish them apart, establishing genetic conservation and selection strategies for Pelibuey, Black Belly and Saint Croix. Pelibuey was determined to be the least diverse population, stressing that taking deliberate action to increase its diversity through higher genetic exchange or importation might be necessary. This study builds a basis and highlights that microsatellite panels and new private alleles are important for a proper genetic identification of animal breeds, and can be developed into more precise standards for an effective Mexican ovine cattle management.

1829 - Partial biochimica and immunological characterization of some pregnancy-associated glycoproteins (PAGs) in ovine

Author: Gerardo Perera-Marín, Griselda Valdez Magaña , Giovanna León-Legaspi, Everardo González-Padilla, Silvia Ivonne Mora Herrera, Rogelio Alonso-Morales, Clara Murcia

Objectives

Pregnancy-associated glycoproteins (PAGs) are synthesized in the placental cells of ruminants and are detectable in blood, milk, and urine. PAGs belong to the aspartic proteinases family and have been described more than 10 different variants, in ruminants vary spatially and temporally during gestation. For instance, in sheep, ovPAG2 expression occurs on day 14, with three additional PAGs identified by day 17, one resembling ovPAG-1 and two unknown ones named ovPAG-10 and ovPAG-11. OvPAG-5 and 7 are identified by day 16, whereas ovPAG-3, 6, 8, and 9 are expressed from day 18 to 22 after conception. The biological role played by this family of glycoproteins is intricate and not entirely elucidated. It is presumed a potential roll in the placenta-uterus interface, acting as molecular bridges. Many of these proteins have been obtained and characterized from placental extracts by precipitation with 80% ammonium sulfate, the possibility to purify novel PAGs by precipitation with other concentration of ammonium sulfate remains unexplored.

The aim of this investigation was to study PAG proteins obtained from extracts of ovine placenta at 100 days of gestation through precipitation with 40% ammonium sulfate.

Material and methods

The fetal cotyledons from sheep placental tissue were homogenized, and then differentially precipitated with 40% (PAG40) and 80% ammonium sulfate (PAG80). Prior to purifying Extract 40 and Extract 80 through ion exchange chromatography, we identify the presence of PAG immunoreactive proteins, for which a Western blotting system was developed using the primary anti-PAG1 antibody. The PAG40 fraction was eluted on DEAE-Sephacel, and the pure protein was recovered with 0.080 M and 0.16 M NaCl, yielding 0.083 and 0.141 mg/g of tissue, respectively.

Results

Both fractions, eluted on DEAE-Sephacel, revealed two predominant proteins of 52.1 kDa and 26.1 kDa. The 52.1 kDa protein showed 100% homology when compared to amino acid residues of isoforms 3 and 4 of the ovine species. The pure fraction eluted in a pH range of 5.64 to 4.22, and two-dimensional (2D) analysis revealed the presence of three mass variants with different isoelectric points.

Conclusions

The results confirmed the viability of recovering PAGs in the protein fraction obtained with a 40% saturation of ammonium sulfate. The 52.1 kDa molecular weight variant exhibits charge heterogeneity and possesses biochemical and immunological characteristics of PAGs.

Buffalo and camelids

1520 - Neurological deficits in an alpaca after microchip placement

Author: Floriana Sajovitz , Michael Leschnik, Sonja Franz, Thomas Wittek

Objectives

A 1.5-months-old male alpaca cria was admitted to the animal hospital (University Clinic for Ruminants, Vetmeduni Vienna) showing severe neurological deficits after microchip insertion. It was pre-reported with cardiovascular insufficiency, low body temperature, an irregular heartbeat and lateral recumbency. It showed no response to initial therapy by the referring veterinarian. At initial examination the cria was in lateral recumbency, showing opisthotonos. However, a manual passive flexion of all limbs was possible. The patient showed a decrease in alertness, a low body condition and cold limbs. The animal was painful in the cervical spine region and the neck muscles were firm on palpation.

Material and methods

Differential blood count and blood chemistry showed leucopenia, hyperglycaemia and indicated a dehydration. Diagnostic imaging (CT) revealed that the microchip was located near the C2 in the muscle. However, no evidence of contusion of the spinal canal was found at the time of the CT scan. The physical examination and the assessment of the cerebrospinal fluid led to the assumption of a septic meningomyelitis due to a damage of the dura mater during the placement of the microchip. Physiotherapy was carried out daily.

Results

The alpaca cria showed a significant and rapid improvement in clinical signs within the first few days of treatment. After three weeks the animal was referred home with mild ataxia and a slight head tremor when stressed. One year later, the alpaca cria shows no clinical deficiencies. It was easily integrated into the herd again. However, it had to be bottle fed as the mother did not accept it afterwards.

Conclusions

This case report will serve as a resource for veterinary practitioners involved in the treatment of similar cases. In general, insertion of microchips is a safe procedure when correctly administered and the animal is fixated properly by a trained and experienced person. In rare cases complications may occur.

1507 - Can limiting stress be a part of mange treatment in alpacas? A case study

Author: Marilena Bolcato , Mariana Roccaro, Arcangelo Gentile, Angelo Peli

Objectives

The term "stress" denotes a set of physiological and behavioral alterations triggered by aversive stimuli, which may encompass chemical or biological agents, or events that induce a stress response. This case report focuses on the clinical presentation and treatment of a mixed mite infestation in three alpacas shortly after their arrival in an educational farm. Particular attention was dedicated to stressor identification and management, which revealed to be crucial for a successful clinical outcome.

Material and methods

Three male alpacas, aged between 5 and 6 months, purchased by a large educational farm located in Northern Italy, were referred for dermatological problems.

Clinical examination, assessment of stress, including stressor identification, behavioural and physiological measures, and parasitological exam were conducted.

Results

At clinical examination, all the alpacas showed mild itching and the presence of alopecic and hyperkeratotic crusty lesions on the ear pinnae, sternum, axillae, belly, medial region of the hind limbs, and perineal region. In all animals, the mucous membranes were pink with a normal refill time. Skin scrapings were obtained from the affected regions of all alpacas, and parasitological exam confirmed the presence of *Sarcoptes* spp.

Subcutaneous ivermectin was administered at the dosage of 0.2mg/kg and the treatment was repeated after 7, 14, and 21 days.

Apart from the typical stressors that are commonly encountered in educational farms, including noise from visitors, music, and children shouting, additional ongoing stressors were recognized. These included injury (caused by the mange), exposure to new experiences (such as interactions with the vet), handling, restraint in both standing and lying positions, and various medical care and procedures.

Throughout each clinical examination, all three alpacas consistently exhibited heightened vigilance, engaged in activities such as running and jumping, attempted to escape handling, and vocalized. Alpaca 3 additionally displayed urination and signs of muscle tension during these evaluations.

The physiological responses observed in all three animals included tachycardia (with rates ranging from 120 to 130 beats per minute) and tachypnea (with rates ranging from 28 to 35 breaths per minute) consistently across multiple days. In the case of alpaca 3, trembling was noted on every occasion. Rectal temperature remained consistently within the normal range for all alpacas, recorded between 38°C and 38.7°C.

At day 14, all animals showed no itching.

At day 21, clinical improvement was evident in alpacas 2 and 3, rare crusty lesions but no hyperkeratosis were detected in alpaca 1. The parasitological exam performed from the previously sampled skin areas showed no presence of *Sarcoptes* spp. and minimal presence of *Chorioptes* spp. Given the improvement of their clinical condition, treatment was suspended, although full recovery had not yet taken place.

A month following the final treatment, the parasitological examination yielded negative results. Three months later, there were no observable clinical symptoms, and full hair regrowth was observed in all animals.

Conclusions

While the impact of stress on human health is widely recognized, there seems to be comparatively less awareness of this issue in the veterinary domain. Stress has been directly

linked to various dysfunctions, encompassing immune system suppression and cutaneous diseases. Furthermore, given that both the skin and the nervous system originate from the embryonic ectoderm, a significant correlation between these two systems is evident. Recognizing and addressing stressors thus constitute a crucial and inherent aspect of therapy, particularly in animal species highly susceptible to stress, such as alpacas.

1222 - Study on composition of alpaca colostrum

Author: Thomas Wittek , Kathrin Rychli, Michael Reichmann, Thiemo Albert, Maria Gaier

Objectives
Colostrum provides the newborn with nutrients and immunoglobulins. Immunoglobulins play an important role for the immune system function in neonatal South American camelids since they are born agammaglobulinemic. A detailed knowledge on the composition of the colostrum and milk is needed for the development of colostrum and milk replacers which are needed if the crias have to be hand-raised. The objectives of the study were to measure the concentrations of immunoglobulin G (IgG), fat, protein, lactose and minerals in alpaca colostrum.
Material and methods
Colostrum samples were obtained daily over four days after parturition from 20 alpaca mares. The IgG concentrations were determined by radial immunodiffusion (RID) using a Camelid IgG Test Kit. Fat, protein, and lactose were determined using classic laboratory methods; mineral concentration have been measured using automatic analyzers (ICP-MS/OES).
Results
The concentrations of fat and lactose increased from day 1 (0.5 %, 4.0 %) to day 4 (5.3 %, 5.0 %), protein decreased from 20.4 % on day 1 to 8.30 % on day 4. The IgG concentration decreased significantly from 26,319 mg/dL on day 1 stepwise to 3849 mg/dL on day 4. Minerals have been sparsely studied in alpaca colostrum in the past; however, many of which had the highest concentrations in colostrum immediately after birth. There were significant correlations between IgG concentration and the other components of the colostrum. While the correlations between IgG and fat ($r = -0.69$, $p < 0.001$) and lactose ($r = -0.64$, $p < 0.001$) were negative, the correlations with protein ($r = 0.91$, $p > 0.001$), magnesium ($r = 0.86$, $p < 0.001$) and cobalt ($r = 0.87$, $p < 0.001$) were strongly positive.
Conclusions
This study contributes to the knowledge on the composition of alpaca colostrum which can be used to develop replacers. Due to the strong association, the colostrum protein concentration could be used as proxy for the IgG content.

Public health, food quality and traceability

1778 - Characteristics of Extended-Spectrum ß-Lactamases-producing Escherichia coli from the Shkumbini River in Albania

Author: Florian Plaku , Ilir Kusi, Erinda Lika, Elena Tsavea, Esmeralda Dushku, Virginia Giantzi, Charalampos Kotzamanidis

Objectives
The objective of our study was to investigate the occurrence, and phenotypic as well as molecular characteristics of extended spectrum β-lactamases producing E. coli (ESBL-Ec) in Shkumbin River. Throughout the sampling period, our primary focus was exclusively directed toward isolating ESBL E. coli in the river.
Material and methods
We collected water from six separate sampling sites, transported and stored it under ideal temperature conditions before analysis within 24 hours. After growth on tryptone bile X-glucuronide agar (TBX) plates containing 4 µg/ml cefotaxime, colonies were carefully selected for a synergy test to confirm the presence of ESBL-Ec. Antimicrobial susceptibility of ESBL-Ec isolates against 15 antimicrobial agents was determined by the standard disc diffusion method on Mueller–Hinton agar. Detection of three virulence genes (eagg, ast, and k99) was performed by multiplex PCR.
Results
Sixteen ESBL-producing E. coli strains were isolated from six of 100 % of river water samples collected from the surface waters of the Shkumbin River. Antimicrobial susceptibility testing of the 16 ESBL-producing strains showed their resistance against meropenem (MEM), ampicillin (AMP), streptomycin (S), and gentamycin (CN). Molecular characterization of the isolates showed that of the sixteen ESBL E. coli strains tested, only one tested positive, indicating the presence of the k99 gene which is known to cause severe neonatal diarrhea in calves and other animals, including lambs and piglets; individual veterinarians reported the occurrence of profuse diarrhea in calves on five farms within the specified geographic region.
Conclusions
Additional research is essential to improve our understanding of the prevalence of ESBL-Ec carrying virulence genes and the potential risks they may pose. Sampling in slaughterhouses is crucial to determine the characteristics of potential ESBL-Ec, highlighting the link between farm animals and aquatic environment.

1171 - Monitoring colostrum quality as a lever for improving calf health

Author: RAPHAEL GUATTEO , Sébastien Geollot, Audrey Brunet, Luc Durel, Thibaut Devambez

Objectives

The transfer of passive immunity is a key factor in the good health, growth future production and reproductive performance of cattle. To improve and facilitate the management of passive immune transfer, different rules or standard operating procedures do exist such as the 5 Q rules (Quality, Quantity, Quickly, Quite clean and Quantifying). The quantification of colostrum quality and its quantification over time needs to be measured systematically and appropriately and should be compared with a target value. However, regarding colostrum quality, in the last decades, the mean observed in different studies, mainly from the US, has tended to be considered as the standard to be attained. Thus, the threshold of 50g/L is often cited as a target value for good-quality colostrum in dairy cows. However, these values are based on studies mostly conducted in a North American context where the genetics and production levels differ from those in Europe. Can and should we be more ambitious when it comes to colostrum quality? Therefore, the main objective was to set up an observatory of colostrum quality, first in France as proof of conformity and then in several European countries. A second objective was to look for putative variation factors of colostrum quality especially around dry period management.

Material and methods

Voluntarily, during the 2020-2021 season, 3794 cow colostrums from 103 French farms (55% suckling, 45% dairy), were measured using an optical refractometer to assess the concentration of IgG in the colostrum. In 2021-2022, the operation was repeated, with 1663 colostrums (72% suckling,28% dairy) from 53 farms at the time of this submission. The farmers also filled out a questionnaire to collect the factors of variability in colostrum quality (e.g. selenium intake, antiparasitic treatment…). A similar collection just began in Romania (n=216), Switzerland (n=203), Ireland (n=207) and Italy (n=296) (collecting is still ongoing). The quality of colostrum was described according to the breed and type of production and parity. In addition, the putative influence of different factors on colostrum quality was investigated using mutlivariable analysis. To our knowledge, this is the largest database on colostrum quality.

Results

Finally, 8918 colostrum from 228 herds concerning 15 breeds were collected and submitted for analysis. The average IgG concentrations were:

- For dairy cows in France: 77.2 ± 45.1 g/L
    - For instance 76 g/L for Prim'Holstein, 69 g/L for Normande and 60 g/L for Montbérliarde breeds
- For beef cows in France: 110.2 ± 48.3 g/L
    - For instance 116 g/L for Rouge des Prés, 113 g/L for Charolaise, 90 g/L for Blonde d'Aquitaine and 84 g/L for Limousine

- For dairy cows in Romania 77 +/- 20 g/L, in Ireland 79 +/- 32 g/L, in Switzerland 57 +/- 43 g/L and in Italy 88 +/- 52 g/L.

Based on these distributions, we propose refining the different thresholds to consider a colostrum good (70 g/L and 105 g/L for dairy and beef respectively) or excellent (105 g/L and 140 g/L for dairy and beef respectively). Interestingly, statistical analysis revealed that primiparous cows were more likely to produce colostrum of better quality than multiparous cows (OR=1.23, [1.03-1.48). Cows for which colostrum was assessed >6h after calving the colostrum quality was lower (OR 0,32, [0.22-0.46]). In addition, cows receiving antiparasitic drugs at drying off had higher IgG values (120.1 vs 90.4 g/L) than those not receiving these drugs ($p<0,001$) as well as those receiving Selenium (97.0 vs 90.0 g/L) ($p <0,01$). Altogether these results confirm that we can and shall be ambitious, for both dairy and beef herds, including primiparous. The results support also the need for optimal dry period management.

Conclusions

The unique and large number of samples, breeds and herds represented makes it possible to move towards creating an observatory that will ultimately allow each breeder to benchmark his or her performance against similar farms. These results should also lead us to revise the thresholds to consider colostrum from moderate to excellent and provide some new insights for an evidence-based approach for the management of the dry period in both dairy and beef cows.

1579 - Risk factors associated with high counts of total plate count and somatic cell count in raw milk and total coliform in fresh cheeses produced in goat family farms.

Author: Israel Daniel Ricardo González, Laura Hernandez Andrade, Edith Rojas Anaya, Gary Gacia Espinosa, Susana Elisa Mendoza Elvira

Objectives

The objective was to characterize and determine the risk factors associated with high levels of Total Plate Count (TPC), and Somatic Cell Count (SCC) in raw goat milk and Total Coliform (TC) in fresh cheeses produced in 7 family goat farms.

Material and methods

Samples of raw milk and fresh goat cheeses were obtained in 7 famlily dairy goat farms in Mexico, three in Querétaro, two in Durango and two in Campeche. A survey of 56 items was applied following the recommendations of the integrated agricultural survey guide (FAO, 2011) considering the following aspects: food and drink, accommodation, milking characteristics, milking site, milking and milk management, sanitary management of animals, production, and marketing of cheese. Samples of raw milk and fresh cheeses made on the farms were taken. The determination of SCC was carried out by flow cytometry. To determine the health indicators, the guidelines of NOM-092-SSA-1994 for TPC and NOM-113-SSA-1994 for TC were followed. The data analysis was carried out using a Fisher's Exact Test ($p<0.05$) to determine the existence of associations between the data obtained in the surveys and the health indicators in raw milk and fresh cheeses. To determine the risk factors, a logistic regression considering the following thresholds for raw milk: TPC: Low (less than 1,000,000 CFU/ml), high (greater than 1,000,000 CFU/ml), SCC: low (less than 1,000,000 cells/mL) and high (greater than 1,000,000 cells/ml), for TC cheeses: high (greater than 300 CFU/g) and low (less than 300 CFU/g).

Results

Of the total raw milk samples evaluated, only one farm from Querétaro and one from the state of Durango remained below the values stipulated by current regulations for TPC, which is 1,200,000 CFU/mL, and for SCC, which is 1,000,000 cell/mL (NMX-F-700-COFOCALEC-2012). In the case of fresh cheeses made from the sampled milk, no farm obtained values below the current regulations for TC, which are up to 100 CFU/g (NOM-243-SSA1-2010). It was possible to determine an association between farm management practices and health indicators ($p<0.05$) and through the logistic regression model the following risk factors (Beta coefficient) for TPC in raw milk were determined: pen cleaning (2.917), type of milking (1.120), washing teats with drinking water (1.120), use of post dip (2.917), for SCC: General cleaning of pens (0.194), washing hands with soap and water (2.10), washing of teats with drinking water (2.10), proper drying of the teats (0.875), use of proper milking technique (2.100), use of post dip(1.556); and for TC: Use post dip (2.917), milk pasteurization (2.917), refrigeration of finished product (0.467).

Conclusions

Both TPC and SCC in raw tank milk and TC are an accurate predictor of microbial contamination in raw goat milk, factors such as: counting with a specific place for milking, washing hands with soap and water before milking, correct drying of the teats,

the use of post dip and the proper handling of milk after milking and during cheese making, are key points to avoid the increase in the microbial load of these products, for which their correct implementation should be widely recommended to improve the quality of the milk with which the cheeses are made in the evaluated farms.

This study was funded by PAPIIT IN203522 and Research Chair CI2216 FES-Cuautitlan UNAM, as well as with self-generated resources from the animal bacteriology laboratory. CENID SAI INIFAP-SADER

1484 - Does bovine raw milk represent a potential risk for vancomycin resistant Enterococci (VRE) transmission?

Author: Elisa Massella , Russo Simone, Rebecca Pezzolato, Anita Filippi, Giorgia Palladini, Chiara Garbarino, Matteo Ricchi, Erika Scaltriti, Roberta Taddei, Camilla Torreggiani, Giovanni Pupillo, Alessio Matteuzzi, Andrea Luppi

Objectives

Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens worldwide, whose transmission is potentially associated with food-producing animal sector. Enterococci are also environmental mastitogenic pathogens, able to colonise mammary glands and be eliminated with milk. VRE infection could represent an occupational disease for breeders and veterinarians and also a risk for people in close contact with animals, especially if immunocompromised. Additionally, new alimentary habits have determined an increase in raw milk and raw milk cheese consumption. The present study aims to evaluate the epidemiological role of bovine raw milk in VRE diffusion and the potential risk to human health.

Material and methods

In the period between December 2022 and December 2023, more than 20.000 individual milk samples and 911 bulk milk samples of bovine origin were analysed. Individual milk samples were screened for mastitogenic bacteria presence. Bulk milk samples included in the study were those evaluated for chemical parameters considered in the Italian payment program based on milk quality.

Bulk tank milk samples were pre-enriched using a selective broth for Enterococci supplied with 20 µg/ml vancomycin and incubated for 48 h at 37°C. Genomic DNA was extracted using a semi-automatic method and a commercial kit. Vancomycin resistance gene (*vanA, vanB, vanC1* and *vanC2/3*) presence was evaluated by performing a multiplex PCR.

Individual milk samples were streaked on Aesculin Blood Agar and incubated for 24-48 h at 37°C. Aesculin fermenting colonies were checked for Gram strain, catalase production and growth on Kanamycin Aesculin Agar (incubated for 24 h at 37°C). Presumptive *Enterococcus* isolates were identified by MALDI-TOF.

Antimicrobial resistance phenotyping was performed with the broth microdilution method for the evaluation of the minimal inhibitory concentration (MIC). The following antimicrobials were tested: vancomycin, teicoplanin, quinupristin/dalfopristin, tetracycline, daptomycin, ciprofloxacin, erythromycin, tigecycline, linezolid, gentamicin, ampicillin e chloramphenicol. MIC results were interpreted referring to the CLSI clinical breakpoints, or when not available, to the EUCAST clinical breakpoints.

Considering vancomycin, strains showing resistance (MIC≥8 µg/ml) were tested for *van* gene presence by multiplex PCR. Finally, the whole genomes of these strains were sequenced (WGS) to deeply analyse their genomic features.

Results

Considering bulk tank milk, *vanC1* and *vanC2/3* genes were detected in 176/911 (19.32%) and 49/911 (5.38%) samples, respectively. Thirty-four/911 (3.73%) samples harboured both *vanC1* and *vanC2/3* genes. *vanA* and *vanB* genes were not detected.

We isolated 134 *Enterococcus* spp. from individual milk. *Enterococcus* strains belonged to 8 different species. The most common were *E. faecium* (91/134, 67.91%) and *E. faecalis* (23/134, 17.16%), followed by *E. hirae* (8/134, 5.97%), *E. cecorum* (4/134, 2.99%), *E. saccharolitycus* (3/134, 2.24%), *E. casseliflavus* (2/134, 1.49%) *E. canintestini* (2/134, 1.49%) and *E. gallinarum* (1/134, 0.75%).

Ninety-three/134 (69.40%) strains were multidrug-resistant (MDR), showing resistance from 3 up to 10 different antimicrobials. Most strains showing resistance to more than five different molecules belonged to *E. faecium* (8/11, 72.73%). The most common resistance was to

ciprofloxacin (99/134, 73.88%), followed by erythromycin (98/134, 73.13%), quinupristin/dalfopristin (78/134, 58.21%), linezolid (55/134, 41.04%), tetracycline (50/134, 37.31%) daptomycin (36/134, 26.87%), chloramphenicol (29/134, 21.64%), tigecycline (11/134, 8.21%), ampicillin (9/134, 6.72%) and gentamycin high level (3/134, 2.24%). No resistance to teicoplanin was observed.

Interestingly, 4 strains showed resistance to vancomycin: 3 *Enterococcus faecium* (MIC = 8µg/ml) and 1 *Enterococcus faecalis* (MIC = 16µg/ml), all carrying *vanC1* gene and multidrug resistant. WGS allowed the confirmation of *van* gene presence, the detection of associated mobile genetic elements and the identification of the complete genetic antimicrobial resistance profile.

Conclusions

Considering these preliminary data, the dairy supply chain doesn't seem involved in the diffusion of vancomycin resistance genes of clinical importance (*vanA* and *vanB*). However, we observed a high prevalence of *vanC* genes in bulk tank milk and also in 4 *Enterococcus* strains associated with mastitis. Despite their lower frequency, *vanC Enterococci* have been reported in several human outbreaks, showing high vancomycin resistance and difficulties in treatment efficacy. Therefore, in these cases, also raw milk and related products should be considered as a potential source of infection. The present study suggests how the screening of *van* genes in bulk and individual milk was useful for VRE monitoring in the dairy supply chain, providing important epidemiological information and better defining the potential risk of VRE transmission through raw milk.

1293 - Challenges of regional control of foot-and-mouth disease: SEACFMD reviewed

Author: Peter Windsor Andrew, Harish Tiwari

Objectives

Explore the challenges of regional Foot-and-Mouth Disease (FMD) control in Pool 1 of the Global FMD Control Strategy, via a structured review of the 25 years of the South-East Asia Foot and Mouth Disease (SEAFMD) then SEACFMD campaign with inclusion of China and other countries, aimed at facilitating regional international alliances for transboundary disease (TAD) control.

Material and methods

The SEAFMD program commenced in 1997 to promote collaborative activities through 6 phased roadmaps, including addition of China to create the SEACFMD campaign, with Phase 6 (2021-2025) proposing an evaluation of the campaign from 1997. The evaluation survey tool used the OECD monitoring and evaluation criteria of: relevance; coherence; effectiveness; efficiency; impact; and sustainability. Data from stakeholder online interviews (n=35), questionnaire survey (n= 58), and face-to-face focus group discussions (n=34) from the 26th SEACFMD Coordinators Meeting in Bali in October 2022, were analysed. As recent GF-TADS programs have included Progressive Control Pathway (PCP) for FMD and the Performance of Veterinary Services (PVS) tools, opinions were sought on whether these had increased the momentum of regional FMD control and further, whether the program had influenced progress in equity including animal welfare and gender issues.

Results

Surveys identified agreement that: expansion of SEACFMD to countries beyond the Greater Mekong Subregion was appropriate due to increasing interconnectedness of FMD virus pools; evolution of the campaign phases had facilitated progress, despite repeated incursions of FMD including new virus strains, plus the emergence of Lumpy Skin Disease (LSD), African Swine Fever (ASF), Peste Petits Ruminants (PPR), and other diseases into the region; and coordination and communication amongst member countries had progressed, although political and financial commitments for sustainability of interventions inclusing vaccination and biosecurity remained insufficient.

Conclusions

The incursion of FMD (O/Ind2001e) into Indonesia after almost a 40-year absence in early 2022 reflects the many challenges that remain and suggest that despite the progress of SEACFMD, progress has failed to adjust to the increading risks resulting from increased movement of livestock and their products. Problems to address include: insufficient biosecurity practices with unregulated animal movements between and within infected countries; difficulties of vaccine resourcing, application and delivery, compromising efficacy; low-level field technical capacity to deliver adequate surveillance and response; low levels of local engagement of smallholders in disease control awareness; lack of national emergency disease response capacities; and ongoing difficulties in coordination of national and international control programs.

Reference: Blacksell SD, Siengsanan-Lamont J, Kamolsiripichaipom S, Gleeson LJ, Windsor PA. (2019) A history of FMD research and control programmes in Southeast Asia: lessons from the past informing the future, Epidemiol Infect, 347:1-13, doi: 10.1017/S0950268819000578

1169 - The effects of calves fed colostrum from dairy cows treated with first generation cephalosporin dry cow treatments on selection and spread of antimicrobial resistance in dairy calves.

Author: D. C. Speksnijder, Q. Lu, S. Sietsma, A. Timmerman, J. M. Swinkels, A. L. Zomer

Objectives

Antimicrobial resistance is a major threat for human and animal health. Especially transmission of Extended Spectrum Beta Lactamase producing Enterobacteriaceae (ESBL-pE) from animals to humans is considered a huge risk for human health. Dairy calves are often found to carry ESBL-pE in their gut from birth, but its source is not yet elucidated. A possible route could be transmission from the dam via faeces or colostrum. First generation cephalosporins (1GC) are globally used in dairy cows for dry cow therapy (DCT) but may have the potential to select for ESBL producing bacteria while this potential is perceived to be lower for narrow spectrum penicillin (NSP). In this study, we compared the effects of a 1GC versus a NSP DCT on selection and spread of cefotaxime resistant (CTX-R) *E. coli* in the gut of the neonatal calf.

Material and methods

On three commercial Dutch dairy farms known to harbour ESBL *E. coli* in their herd, cows before drying off were screened for CTX-R resistant *E. coli* in their faeces using selective enrichment of 1mg/ml CTX in LB broth followed by plating on MacConkey agar. ESBL *E. coli* positive cows were randomized to receive standard NSP DCT (600mg cloxacillin) or 1GC DCT (300mg cefapirin) at drying off. Post- calving, calves were fed their dams' colostrum for 2-3 days. Faecal samples of the neonatal calves born from these cows were taken at day 2, day 7 and day 14 after birth. Samples were stored under cooled conditions (2-8 ^{o}C) and send to the laboratory within 48 hours.

Faeces was enriched in LB broth with 1mg/ml CTX, incubated overnight and plated on MacConkey agar for screening of the presence of CTX resistant *E. coli*. Confirmation of *E. coli* occurred with MALDI-TOF. If positive, quantitative analyses was performed in order to calculate CTX-R *E. coli* CFU/gram faeces. Fresh faeces was mixed with saline and 10-fold dilutions were plated on MacConkey + CTX and incubated overnight for quantitative counts. As the total CTX-R *E. coli* counts were not normally distributed, the one-way ANCOVA test was used to assess whether a difference in counts between the two groups could be observed with time points of sampling as covariate and farms as random factor.

Results

A total of 172 cows were screened for the presence of CTX-R *E. coli* in their faeces before drying off. 22 cows (13%) tested positive on CTX-R *E. coli* and were included in the study of which half received 1GC and others NSP DCT.

Preliminary results from the samples taken from calves (n=10 in each study arm) indicated mean CTX-R *E. coli* counts of $3.73*10^6$ (SD $6.52*10^6$) and $2.20*10^6$ (SD $4.14*10^6$) CFU/gr faeces on day 2, $8.36*10^6$ (SD $2.53*10^7$) and $3.44*10^5$ (SD $8.47*10^5$) CFU/gr faeces on day 7 and $1.73*10^7$ (SD $3.75*10^7$) and $4.91*10^6$ (SD $1.00*10^7$) CFU/gr faeces on day 14 in the cefapirin and cloxacillin group respectively. On day 2, all calves harboured CTX-R *E. coli*; on day 7 CTX-R *E. coli* could not be found in one calf in the 1GC group and in three calves of the NSP group; on day 14 CTX-R *E. coli* could not be found in one calf in the 1GC group.

Although on each sampling moment the quantitative counts of CTX-R *E. coli* were on average higher in calves born from dams that had received 1GC, no significant differences in CFU counts per gram faeces could be observed compared to calves born from dams receiving cloxacillin DCT in the total study period ($F=2.401$; $p=0.20$) when adjusted for farm and sampling time point. Further analysis based on Whole Genome Sequencing of the isolated CTX-R *E. coli* is pending.

Conclusions

Based on our preliminary results of a worst-case scenario study within a limited number of animals, faecal CTX-R *E. coli* counts in neonatal calves born from dams harbouring CTX-R *E. coli* in their gastrointestinal tract around the time of drying off did not significantly differ between groups of dairy cows receiving cloxacillin or cefapirin DCT. Huge variations in faecal CTX-R *E. coli* counts were found in calves within both study groups, indicating that other cow or farm related factors are possibly of greater influence for selection and transmission of ESBL-pE via the calf.

Animal behavior and welfare

1828 - Identifying animal-based measures to assess dairy cattle welfare in Indonesia: A Delphi study

Author: Istianah Maryam Jamilah, Dyah Ayu Widiasih, Richard Anthony Laven

Objectives
A systematic animal welfare assessment is useful for identifying current welfare status, particularly whether the proportion of farms meets specific standards. As dairy cow systems and problems vary significantly worldwide, such welfare assessments need to be system-specific, practical, and achievable within the system they will be used in. This study aimed to identify potential animal-based measures to include in a dairy cattle welfare assessment protocol for Indonesian dairy farms.
Material and methods
A Delphi consultation, with two rounds of e-survey, was used to reach a consensus of expert opinion. Four organizations associated with dairy cattle welfare in Indonesia were contacted to identify experts who would be useful in the survey. The 63 respondents who were identified then sent the first e-survey asking them to list their three key welfare problems for Indonesian dairy farms and to two welfare measures per problem that could be used to quantify the problem. Experts who responded to the first survey were sent the second e-survey four weeks later. This asked the expert to rate the importance of the measure suggested during the e-survey 1 on a five-point Likert scale and to list their top five most important measures. The maximum possible scores of the importance of all measures suggested from the e-survey 2 were calculated to identify potential measures to be included in the welfare assessment protocol.
Results
Forty-seven experts responded to the e-survey 1 and identified 55 welfare problems and 83 animal-based measures. Forty-four of those experts responded to e-survey 2, they officially worked as veterinarians (38), academics (5), and professionals working as consultants and businessmen in the dairy cattle industry (1). Based on their responses, we identified 20 key welfare measures with >85% maximum possible score for its importance. These suggested measures were biased towards negative health effects, probably due to the dominance of vets (38/44) in the final panel.
Conclusions
This study identified key health issues on Indonesian dairy farms, developed a welfare assessment protocol, and highlighted a bias in veterinarians' thinking towards negative health effects rather than holistic welfare. Therefore, veterinary understanding and farmer's attitudes should be considered to improve dairy cattle welfare and developing welfare assessment protocols.

1095 - Evaluation of differences in pooled sample metabolic profile results of Flemish farms with high versus low yearly cow mortality rates

Author: Angelique Rijpert , Koen De Bleecker, Pieter Taelman, Ludivine Tillière, Jozefien Callens, Johan Schaep, Robert John Vansaun

Objectives

Monitoring metabolic health of dairy cows using pooled serum sample metabolic profiling (PSM) has been well described[1,2]. PSM, including 21 biochemical markers, was commercially introduced in Flanders (Belgium) by Animal Health Care Flanders (DGZ) in October 2020. Furthermore, DGZ developed an interpretation tool which presents results in a web diagram containing 8 metabolic key areas (Metabolic Scan). Results can be used to identify at risk areas on farms. The study objective was to investigate possible correlations between PSM key area results and mortality rates on Flemish dairy farms submitting PSM in 2022.

Material and methods

A retrospective analysis was carried out on 364 submissions from 311 Flemish farms submitted in 2022 and included 320, 287, 251 PSM's of close-up (21-3 days precalving), fresh: 3-21 days in milk (DIM), and peak lactation cows: 60-120 DIM, respectively. Methods for collecting and storing samples were scrupulously respected. Evaluation included pool results and corresponding percentages of abnormal values per pool, calculated according to van Saun's standard, for the following markers: albumin, alpha, beta and gamma globulins, total protein (TP), beta hydroxybutyrate (BHB), calcium, chloride, creatinine, phosphorus, magnesium, sodium, potassium, non-esterified-fatty-acids (NEFA), selenium, urea, vitamin E, copper, zinc, gamma glutamyl transferase (GGT) and beta-carotene[1,2]. Based on biological significance for interpretation, individual marker results were assigned to 8 metabolic key areas: Ketosis-negative energy balance (NEB-ketosis): BHB, NEFA and GGT; water intake: chloride and sodium; liver function: GGT and urea; trace mineral status: copper and zinc; macro minerals: calcium, magnesium, phosphorus; antioxidants: vitamin E, selenium and beta-carotene; inflammation: alpha and gamma globulins and TP; dry matter intake (DMI): albumin and urea. Subsequently, results were converted into a percentage of abnormal values for each key area.

Furthermore, herd demographics were collected from the Belgian Sanitel database including average number of cows > 2 years and youngstock < 2 years and the total number of cow deaths, culled cows and dead youngstock per submitting farm in 2022. Statistical analysis was performed using JMP computational software (version 16, SAS Institute Inc.). Descriptives statistics were performed on culling and mortality rates. Correlation coefficients were established between the culling and mortality metrics. Farms were categorized into lower than median 0-4% (LM) or higher than median >4% (HM) mortality of cows > 2 years. HM was evaluated in comparison with LM for the PSM key area results. Significance was defined as $p < 0.05$, tendency for $p = 0.05$-0.10.

Results

Median (range) of culling, cow mortality and youngstock mortality rate was 18% (0-52%), 4% (0-38%) and 4% (0-19%), respectively. Culling rate was correlated with both cow (r=0.11, P=.04) and youngstock (r=0.13, P=.02) mortality rates. Correlation coefficient between cow and youngstock mortality rate was 0.28 (P<.001). HM showed a significantly higher mean percentage of abnormal values for the key area water intake in the close-up cow results versus LM (39% versus 31%). In the fresh cow results HM showed significantly more abnormal values for liver function (24% versus 18%) and inflammation (29% versus 24%) and tendencies for more abnormal values for antioxidants (37% versus 32%) and macrominerals (17% versus 15%). At peak lactation in HM category significantly higher percentages were found for inflammation

(22% versus 13%) and dry matter intake (18% versus 10%) and a tendency for a lower percentage of ketosis-NEB (25%-32%).

Conclusions

Results show a wide range of culling, cow and youngstock mortality rates in the Flemish herds that submitted for PSM in 2022. Correlations between them could indicate that overall herd health and farm management are mutual risk factors. HM category results showed more room for improvement specifically in fresh cow period and, to a lesser extent, during peak lactation and close-up than LM category. Furthermore, the results indicate that farms with high cow mortality rates need to consider extra care to reduce inflammation events in early lactation; to improve liver function in fresh cows and to address water and feed availability in general. Therefore, authors conclude that PSM is a valuable tool to identify and improve compromised animal welfare.

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1179 - A delphi study to build pain scoring scale in cattle

Author: RAPHAEL GUATTEO , May Morin, Edouard Timsit, Philippe Gisbert, Damien Remmy, Gwenola Touzot-Jourde

Objectives

Detecting pain in cattle is a prerequisite for good health and welfare management. Until recently, there was no specific pain detection scale for cattle beyond lameness scoring. More recently, some grids have been developed for cattle: some specific to surgery (BOTUCATU grid from De Oliveira et al., 2014) and others not specific to a particular disease (Gleerup's pain scale from Gleerup et al., 2015). In addition, although the use of analgesics seems to be increasing, there is a need for greater awareness of the subject among farmers and veterinarians. Delphi method is an interactive forecasting method which relies on a panel of experts and can be used to help reach expert consensus and develop professional Therefore, the objective of this study was, through a Delphi method, to propose simple and if possible specific grids for detecting pain in cattle in the context of specific diseases.

Material and methods

After a survey of existing grids, all the signs of pain in cattle recognised in the literature were listed. The validation and combination of the signs of interest were carried out based on the Delphi method, using a panel of 12 experts recognised for their expertise in cattle pain. A first round of the questionnaire was submitted, based on a list of possible signs for each of the following conditions: mastitis, metritis, reticuloperitonitis, bovine respiratory disorders, dystocia, normal calving, c-section and dehorning, eye disease, omphalitis, diarrhoea. The experts were asked to answer whether or not the signs included in the questionnaire were relevant, and if so specific, to detect pain. After the first round (11 experts' responses), the criteria considered relevant by 10 experts at least were conserved for the second round. Experts also could add other signs not listed. The second round aimed to consolidate the results of the first round and investigate the relevance of additional signs identified by experts. At this stage, the criteria considered as relevant by 11 experts out of 12 were conserved for the last round which aimed to prioritize the signs and indicate if painkillers should be used when a single sign was detected or a certain number has to be detected.

The pain grids were created thanks to the experts' high level of approval for many criteria. Groupings of signs by clinical similarity were made to reduce the long lists that could be dissuasive for the evaluators.

Results

For instance, for metritis, the relevant signs finally included in the grid were: discomfort on transrectal palpation of the uterus, contracted abdomen, increased lying time, stranguria, and rapid tail movements.

For calf diarrhoea, the relevant signs finally included in the grid were: tight abdomen, tenesmus, tooth grinding, tail flicking, and modification of lying bouts and hind legs position. The different grids for each condition will be presented.

Although combining several signs and conditions could increase the grids' specificity, the grid's sensitivity seems to be favoured by the experts who recommend the administration of NSAIDs as soon as a sign of pain is present: doubt must benefit the animal.

Conclusions

The grids thus developed through the Delphi method will serve as daily support for farmers and veterinarians to improve the detection and management of pain in cattle. The further steps consist of developing an application for smartphones and tablets for both vets and farmers to test these grids in the field and increase awareness around pain management in cattle.

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1312 - Describing behaviors of beef bulls during the pre-breeding period using remote monitoring technology and machine learning algorithms

Author: Vinicius Camargo, Edmond Pajor, Sayeh Bayat, Jennifer Pearson

Objectives

Behavior is a factor that can explain reproductive efficiency and be used for early detection of diseases and injuries in cattle. However, direct observation of beef bulls' behavior can be time-consuming and impractical. Therefore, developing and validating technologies for remote monitoring of bulls is paramount. The objective was to develop machine-learning models to predict behaviors of beef bulls from accelerometer data, assess their predictive performance, and describe behaviors of beef bulls.

Material and methods

Collars with 3-axis accelerometer sensors (Lotek LiteTrack-LR) were deployed on 31 bulls (30 Angus, and 1 Hereford, aged 1.5-6 years old) between April and June over three years (2020, 2021, and 2023). Bulls were restrained in a chute and the cranial portion of their necks were measured to adjust and place the collars. Breeding soundness evaluations were performed, and DNA samples of bulls were collected. The accelerometer was set to a sampling rate of 0.5 Hz in 2020 and 2021 and 1 Hz in 2023. Videos of bulls in pre-breeding pens were recorded during daylight for 30 continuous minutes several times a day framing multiple bulls (48 hours in 2020-2021 and 70 hours in 2023) and analyzed using behaviour analysis software (Boris v.7) to create labels of mutually exclusive behaviors for developing the machine learning model. Collars were removed after an average of 13±0.8 days for each year. Behaviors reported were grazing (GR), resting (standing or lying, RE), ruminating (RU), and walking (WA). One dataset for 2020 and 2021, and another for 2023 were created (due to the sampling rate differences) by merging behavioral observations with the subset of raw accelerometer data with corresponding timestamps from the video data. Secondary labels of activity (Ac), corresponding to behaviors of GR and WA, and non-activity (Na), corresponding to behaviors of RE and RU, were created from the dataset. All processes were performed in Python. The datasets were segmented into epochs of 20 consecutive seconds. A total of 133 time and frequency domain features were extracted from each epoch, and a feature selection process using extra-tree classifiers with a threshold of 0.01 for feature importance was implemented. Hierarchical XGBoost classifier models were created to make predictions on two levels: (1) distinguishing between Ac and Na, and (2) categorizing Ac into GR, WA, and Na into RE or RU. For each level, an average of 16±3 features tailored to the classification task was selected. The leave-one-animal-out cross-validation was used to assess the predictive performance of the models. Final models were trained over the entire datasets to make predictions of behaviors from the accelerometer data to compute 24-hour time-budgets for each bull.

Results

The model created for 2020-2021 had a Matthew's Correlation Coefficient (MCC) of 0.80±0.24 for AC vs NA and 0.61±0.22 for GR, RE, RU, and WA. The precision and sensitivity were respectively 0.93±0.06 and 0.96±0.03 for GR, 0.83±0.19 and 0.88±0.1 for RE, 0.87±0.12 and 0.78±0.21 for RU, 0.74±0.3 and 0.55±0.23 for WA. The model created for the 2023 data had an MCC of 0.98±0.01 for AC vs NA and 0.92±0.02 for GR, RE, RU, and WA. The precision and sensitivity were respectively 0.97±0.02 and 0.98±0.02 for GR, 0.95±0.04 and 0.94±0.03 for RE, 0.93±0.03 and 0.94±0.06 for RU, 0.83±0.12 and 0.67±0.2 for WA. In 2020 and 2021, bulls spent an average of 25% (range: 19%-32%) of their time being active, of which 16% (8%-28%) was spent GR, and 9% (3%-17%) on WA, and 75% (62%-81%) being inactive of which 36% (30%-44%) was spent RE, and 39% (25%-44%) RU. In 2023, bulls spent an average of 27% (21%-31%) of their time being active, of which 22% (17%-26%) was spent GR and 4% (3%-6%) was spent WA, and 73% (69%-79%) of time inactive of which 36% (30%-44%) was spent RE and 38% (27%-42%) RU.

Conclusions

Bulls spent most of their time during the pre-breeding period being inactive. Although the predictive performance of GR, RU, and RE were fairly high, WA could be improved by increasing observations of this behavior in the dataset. Overall, models created from data sampled at 1Hz were more robust than those created from data sampled at 0.5Hz. Therefore, this technology will be used to further investigate the association of behaviors with reproductive performance and early detection of negative health events in beef bulls.

1319 - Use of an automated behavior monitoring system for heat stress to check and optimize cooling strategies

Author: Laura Elvira, Juan Pedro Campillo Beneitez, Alberto Fernández Rodríguez, Juan José Nuñez Casas, Carolina Tejero, Arantxa Villagrá García, Fernando Estellés

Objectives

Heat stress in dairy cows can have detrimental effects on milk production and reproduction, but also in the colostrum quality and the future calf born penalizing its body weight, survival rate and milk production (Dahl y col., 2020; Dahl, 2018; Tao y Dahl, 2013). Traditional indicators like the temperature-humidity index (THI) provide an estimate of potential stress but fail to measure the actual impact on the animals. To address this, the study utilized an automated behavior monitoring system (SenseHub™ Dairy, MSD Animal Health), which records panting, a direct indicator of heat stress (Ramón et al., 2021; Bar y col., 2018; Dahl, 2018), along with intake and rumination data. The objectives of this work were to evaluate the impact of heat stress on cows in different production phases and assess the effectiveness of cooling strategies such as showers.

Material and methods

The study monitored 77 cows from various production cycles, including end lactating (less than 73 days to dry off), dry, and postpartum cows, using a behavior monitoring neck sensor (SenseHub™ Monitoring Neck Tag, MSD Animal Health). Information on panting, eating, and rumination behaviors was collected from June to December 2022 over 24 hours, as well as daily and monthly averages. For lactating cows, two cooling strategies were compared: 2 hours per day in three showers vs. 4 hours per day in six showers. Dry and postpartum cows received 4 hours of cooling per day in six showers.

Results

The results showed that lactating cows experienced the highest impact of heat stress, while dry cows were less affected. The 24 hours monitoring revealed a significant difference between day and night impacts, with the highest panting percentage occurring during the day for lactating cows. In addition, the number of showers did not affect to dry cows as expected. Panting duration correlated inversely with eating, rumination and activity behaviors, indicating the severity of heat stress.

The beneficial effect of cooling on heat stress was clear during the day, but some doubts appear during the night. The average percentage of panting animals varied depending on whether they were in the cooling period or not. Comparing the two cooling strategies for lactating cows, no significant biological differences were observed. Thus, the additional resources (water and energy) and labor doesn't look to be justified.

Conclusions

In conclusion, the monitoring system used in this work proved to be a valuable tool for evaluating heat stress impacts and optimizing cooling strategies. Moreover, the objective data based on the cow response 24 hours per day allowed a real time decision making and give some insights to improve the future cooling strategies.

1332 - A glimpse into tail tip lesions in dairy cows – have we missed something?

Author: Lea M. Lorenz, Kathrin M. Abel, Christine Schmidt, Prisca V. Kremer-Rücker, Mirjam Lechner

Objectives
Tail tip lesions have been observed in fattening cattle with prevalences varying from 35 % to 76 %. These lesions include hairless areas of the tail tip, laceration and / or infection leading to inflammation of larger parts of the tail as well as necrosis. This often results not only in economic loss but has also adverse effects on animal health and welfare. While there are several studies on this issue in fattening cattle, data on tail tip lesions in dairy cows are scarce. However, own previous studies have shown that a variety of lesions on the tip of the tail also occur in dairy cows. The objective of this study was to investigate the prevalence of tail tip lesions in dairy cows.
Material and methods
A developed overview of different tail tip lesions in dairy cows and their manifestations was used to examine cows on six different German dairy farms. After shaving the tail in the region of the switch, cows were examined for the prevalence of 1) lesions on the very tip of the tail, 2) annular lesions, 3) hyperkeratosis, 4) swelling, 5) axis deviation, 6) thinning or 7) fibroma on the distal part of the tail. Examination was performed by one single person. All cows were kept in loose housing conditions on slatted or solid floors, with or without automatic scrapers. Herd sizes varied between 51 and 162 cows. A total of 500 Simmental or Holstein Friesian dairy cows were examined. To calculate the prevalence per lesion, the number of animals affected by the corresponding lesion or lesion manifestation was divided by the total number of animals examined. Mean prevalences were calculated per farm and for the total sample.
Results
Only one cow, out of all 500 examined cows, was free from the above mentioned lesions. 499 animals, however, were affected by at least one lesion. The most prevalent lesions were hyperkeratosis, ranging from 72.9 % to 97.3 % between herds (mean prevalence = 85.1 % ± 7.9) and swelling, with prevalences between 54.2 % and 95.9 % (mean prevalence = 73.4 % ± 18.3). Every lesion could be observed in every herd. The most severe manifestations of lesions at the very tip of the tail, according to our previously established overview, are bleeding or necrosis up to part loss of the distal part of the tail. We found bleeding or necrotic tail tips in four of the six farms, with prevalences ranging from 0.66 % to 26.03 % (mean prevalence = 8.36 % ± 11,39).
Conclusions
To our knowledge, this is the first study to show prevalences of different tail tip lesions in Simmental and Holstein Friesian cows on German dairy farms. The high prevalence of the lesions in all farms evaluated, under different housing conditions and in both breeds, and the occurrence of severe manifestations such as bleeding or necrotic tail tips, may indicate an animal welfare problem that has apparently gone unnoticed until now. Further research is needed to assess whether this phenomenon occurs on other farms and in different regions and countries as well, and to elucidate the aetiology, pathogenesis and risk factors of the lesions observed.

1398 - Assessment Welfare protocol to a buffalo farm

Author: Melina Yasuoka Marie

Objectives

This summary aims to contribute expertise in the welfare evaluation of buffalos, and adept at conducting comprehensive assessments and implementing welfare enhancement strategies.

Material and methods

There were evaluated 1160 Murrah buffalos on a farm located at Brotas, São Paulo, Brazil. The body condition score (BCS) assessment was identified with the animals brought from lots of farm paddocks, inspected one by one, contained 4-5 animals in the trunk hose/trough in single file, individually marked with a stick and paint, photographed and counted, and at the end of each batch, the animals returned to their paddock to start the next batch. The BCS methodology used was according to Alapati and collaborators (2010), Welfare Quality (2009) and De Rosa et al.(2007).

The animals evaluated were separated in 6 lots (males, before calving, mixed lot, new maternity (calves 30-90 days), old maternity (calves at weaning to pubertal), and after calving). The feeding points and environment were evaluated by inspecting the feeders and drinker, paddocks, and indicated by current managers. And health and behavior assessment were identified with the animals brought from lots of farm paddocks, inspected one by one, contained 4-5 animals in the trunk hose/breech in single file, individually marked with crayon stick, photographed and counted, and at the end of each batch the animals returned to their paddock to start the next batch.

Results

The protocol proposes the final diagnosis of well-being on a scale of five degrees: very high, high, regular, low and very low, using a simplified form of integration. The final conclusion for a very low level of well-being will be defined when three or more sets of indicators are classified as inadequate or when there is intentional physical aggression; a low level of well-being will be determined when there is an inadequacy of one or two sets of indicators; regular level of well-being when two or more sets of indicators are considered regular and none is considered inadequate, high level of well-being when only one set of indicators is regular and very high level of well-being when all sets are adequate of indicators (DE ROSA,et al., 2007).

Was observed that all groups with the exception of the “after calving” lot, the others groups have a satisfactory level of well-being on the farm, the batch of recently calved animals had low body scores (BCS 1 and 2), the feeders were full of food and water, but as long as the animals have this low score, the diet needs to be adjusted to meet the animal's demands during this period. Drying very thin cows is of great importance as it reduces one of the energy demand pathways. Carry out artificial feeding to ensure the health of calves with dry mothers. The environment was extremely humid, without any grass, disqualifying the place for postpartum cows and newborn calves, considering that their immunity is low.

Conclusions

Assessing buffalo welfare involves considering various factors, including their physical health, behavioral needs, living conditions, and the overall management practices. Here are some key aspects to consider in buffalo welfare evaluation: 1.Ensure that buffalos receive a balanced and adequate diet to their nutritional requirements. Access to clean water and appropriate feed is crucial; 2.Evaluate the living conditions of buffalos, including the availability of shelter and protection from extreme weather conditions. Adequate space, proper ventilation, and comfortable resting areas are essential; 3. Regular veterinary care for maintaining buffalo health. Monitoring and addressing any signs of illness promptly is essential. Vaccinations and disease prevention measures should be in place; 4.Buffalos has natural behaviors that need to

be considered. They should have enough space to exhibit natural behaviors such as grazing, socializing, and resting. Enrichment activities can also contribute to their well-being; 5.If buffalos are in reproduction programmer, it's important to ensure that breeding practices are ethical and considerate of the animals' well-being. Monitoring the health of calves and providing appropriate care is essential; 6.Buffalos are social animals, and their well-being is influenced by social interactions. Ensuring that they are kept in compatible groups and minimizing social stress is important (BROOM & MOLENTO, 2004; DE ROSA, ,et al., 2005, 2007; WELFARE QUALITY, 2009; AMARAL, et al., 2019).

1700 - Comparative analysis of hair cortisol concentrations from different body regions and coat color in dairy goats

Author: MAYRA SIERRA GARCÍA, IRMA EUGENIA CANDANOSA ARANDA

Objectives

Analyze and determine the influence of body region and coat color on hair cortisol concentrations in stabled dairy goats.

Material and methods

From a group of 31 multiparous, non-pregnant, non-lactating stabled dairy goats of Alpine French, Toggenburg, and Saanen crosses, over 4 years old, body weight of 56.45 ± 1.48 kg, and clinically healthy. Ten goats were randomly selected for hair shaving from six body regions (head, neck, chest, side, hip, and tail tip). The samples were categorized by hair color (light, medium, and dark) and stored in waxed bags at room temperature until processing. To determine hair cortisol concentrations (HCC), an adaptation of the cortisol extraction methodology proposed by Davenport *et al*. (2006) and Koren *et al*. (2002) was applied. Cortisol determination was carried out using the enzyme-linked immunosorbent assay (ELISA indirect) method with the Arbor Assays Cortisol commercial kit (K003-H1/H5) DetectX. The intra-assay and inter-assay coefficients of variation (CV) were 8.76% and 8.13%, respectively.

Data analysis employed SASâ software. The study factors were body region and coat color, with hair cortisol (pg/mg) as the response variable. The Shapiro-Wilk normality test and Bartlett's test for homogeneity of variances were used. ANOVA (Proc GLM) based on a completely random design was applied, followed by a Tukey test to assess differences between body regions and coat colors. Statistical differences were considered $p < 0.05$.

Results

A significant influence of hair cortisol concentrations (HCC) in dairy goats was evident across different body regions ($p < 0.05$). The lowest HCC was observed in the head region (15.62±1.10 pg/mg) and chest region (15.44±1.54 pg/mg), while the highest concentrations were detected in the side region (42.78±4.85 pg/mg) and hip region (46.28±3.53 pg/mg). Additionally, HCC in the neck and tail regions were 35.42±2.44 pg/mg and 28.07±3.95 pg/mg, respectively.

Regarding coat color, lower HCC was found in dark-colored hair ($p < 0.05$) in the side region (25.99±8.11 pg/mg) and hip region (32.01±5.73 pg/mg) compared to light and medium-toned coats. In the tail region, a significantly higher concentration ($p < 0.05$) of HCC was observed in medium-toned hair (33.53±3.63 pg/mg) compared to light (20.05±8.11 pg/mg) and dark (23.25±4.05 pg/mg) hair. No significant differences ($p > 0.05$) were identified in HCC in the head, chest, and neck body regions based on coat color.

Conclusions

Hair cortisol concentrations in dairy goats highlight the significant influence of body regions and coat color on stress response. Lower concentrations in the head and chest, contrasted with elevated levels in the side and hip regions, reveal variability in stress management. These results underscore the importance of considering anatomical and phenotypic factors when assessing welfare in dairy goats. Future research should explore underlying mechanisms and validate these findings in diverse conditions and populations to enhance their applicability. This study contributes to the knowledge of animal welfare, emphasizing the need to address the complexity of stress response in goat production.

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Socio-economic aspects of ruminant production

1192 - Economic and performing benefits of implementing an automated behavior monitoring system in a Brazilian Dairy farm

Author: Anna Luiza Belli, Juan Pedro Campillo Beneitez, Brenda Barcelos, Patricia Roberta Salles

Objectives

The economic benefits of adopting an automated behavior monitoring system (ABMS) were evaluated on a commercial dairy farm in Brazil by measuring changes in reproductive and health performance parameters. Dairy farms must be efficient in order to remain profitable. Technologies impact that by enhancing efficiency, economics, decision making, animal well-being and peace of mind for the producer. Hence, Automated Behavior Monitoring Systems (ABMS) must have positive impacts leading to the economical and performance efficiency.

Material and methods

In July of 2022, a Brazilian dairy farm milking 170 cross bred cows (Gyr x Holstein) adopted the ABMS (SenseHub™ Dairy, MSD Animal Health). At installation, cows in prepartum until further in lactation were wearing behavior monitoring neck tags (SenseHub™ Monitoring Neck Tag, MSD Animal Health) that collected information on animal behavior. This information was transmitted to a controller and transformed by algorithms into insights that can be used to make decisions regarding health, reproduction, nutrition and well-being. The total investment in the ABMS included the acquisition of 110 tags, software, warranties, installation services and long-term technical support. Prior to adopting the ABMS, the farm used only Timed Artificial Insemination (TAI) for breeding animals, and cows were visually checked to identify potential problems. Once the ABMS was installed, the system identified animals in heat and system alerts were utilized to flag animals requiring attention, particularly for screening post-partum animals that needed attention. The economic impact of adopting the system was assessed by considering various factors, including the number of TAI protocols, reproductive indexes (days open (DO), pregnancy rate (PR), insemination rate (IR), conception rate (CR)); the average milk yield per cow, and the percentage of adult animal mortality. A comparison of the results from one-year pre-system with one-year post-system was performed. Data collected from August 2021 to July 2022 constituted the pre-system period. Data from August 2022 to August 2023 represented the post-system period. Only data from lactating cows were utilized in the analysis, and no other relevant management changes were implemented on the farm during the evaluated timeframe.

Results

The results showed an increase of 6% in IR, 29,1% in CR (34.7 and 44.8%) and 35.9% in PR (17.8 and 24.2% prior and post system respectively) after implementing the ABMS. Adopting the calculations from the database of a management evaluation economic program (*ReproMoney®* Program, University of Wisconsin), the increase in the PR from 18% to 24% led to an annual net revenue of US$100,00 per cow. Considering that the farm has an average of 170 lactating cows per year, the total revenue regarding the increase in the PR is US$17.000,00. Additionally, the number of TAI protocols performed decreased from 750 to 373, resulting in a cost savings of US$2,626.76. The cost of the day open informed by the farm is U$7,00. Therefore, the reduction of 15 in DO led to an economy of US$105,09 per cow per year and a total of US$17.865,39 considering the 170 cows in lactation. Regarding health there was a 2% decrease in mortality The average value of a cross bred lactating cow sold by this farm in the Brazilian market is US$3.018,10. The reduction of 2% in the mortality of 170 cows, corresponded to at least 3 less

deaths in one year and an economy of at least US$9.054,32. The average milk yield per cow increased from 31.7 liters in August 2022 to 32.6 liters in August 2023.

Conclusions

When accounting all the economic benefits provided to the farm by the adoption of the ABMS, the total income generated by the technology in one year was US$43.919,71. The ABMS is a technology that improves not just the performance of the dairy business, but also enhances the quality of life for the farmer and the animal well-being by providing efficient tools to monitor the process of the activity.

1152 - Economic benefits of abrupt dry-off with cabergoline injection in dairy cows. A field case in Vietnam

Author: Philippe Gisbert, Tung Nguyen, Carla Azevedo, Juan Munoz-Bielsa, Tam Nguyen Minh

Objectives

In order to dry-off dairy cows with the lowest possible milk yield at the time of the last milking, farmers often resort to gradual dry-off by reducing feed intake and/or milking frequency during the last weeks of lactation.
Drying-off abruptly, i.e. stopping milking without first reducing feed intake or milking frequency, makes it possible to limit the workload and optimize the animals' milk production. However, this can lead to an increase in udder pressure particularly 24-48 hours after the last milking. This causes discomfort for the cow and milk leakage, linked to the development of new intramammary infections at dry-off[1]. The use of cabergoline (Velactis®) just after the last milking helps to overcome these negative aspects of abrupt dry-off.
This study aimed to evaluate the reduction of milk leakage and the economic value of Velactis® in terms of milk production gains on a commercial dairy farm in Vietnam under field conditions.

Material and methods

The study was carried out on the Nutimilk farm located in a semi-mountainous area of central Vietnam. 97 Holstein cows were included. The cows were randomly assigned to two groups: cows in Velactis group (n=41) remained on the same feed ration until the last milking, and received an injection of Cabergoline 5.6 mg (5mL, Velactis®, Ceva Santé Animale, France) given intramuscularly just after the last milking. Cows in control group (n=56) were dried-off according to the farm's usual procedure: switching from the lactation ration to the dry cow ration one week before the day of dry-off, and housing with cows already dried. After the last milking, animals from both groups received dry-cow therapy and were grouped together in the dry-cow barn.
Milk production for the last 7 days of lactation was collected from the farm's production management software. In addition, milk leakage was assessed two times: once 24 hours and once 48 hours after last milking using an inspection mirror placed under each teat. Milk leakage was considered to have occurred when a stream of milk emerged from one or several teats, milk drops were visible at the end of at least one teat, or milk was observed on the floor beneath the udder.

Results

Average production over the last 7 days of lactation was 103.48 kg milk per cow in the control group and 152.22 kg milk per cow in the Velactis group. Cows that dried-off abruptly therefore produced 48.74 liters more milk than cows progressively dried-off.
The price of milk in Vietnam is 1,500,000 VND (around 64 USD) per 100 liters. The value of the extra production is 48.74/100 * 1,500,000 = 731,100 VND. As a dose of Velactis® is sold for 530,000, the profit is VND 201,100 per cow (around 8.25 USD). However, other costs such as the difference in feed costs and labor costs could not be estimated in this study.
There was no significant difference between the two groups in milk leakage at 24h and 48h (p=0.13 and p=0.39 respectively, Fisher exact test). This shows that abrupt dry-off with Velactis® delivers the same benefit in reducing milk leakage than gradual dry-off. In contrast, published data comparing abrupt dry-off with and without cabergoline showed an 82% reduction in the risk of milk leakage in favor of treated animals[2].

Conclusions

This field study demonstrated the economic benefits of the systematic use of Velactis® under Vietnamese breeding conditions: 48 kg additional milk produced for the last 7 days and net profit gain of 8.25 USD per cow. A more detailed economic study incorporating differences in feeding

costs, labor costs and the economic impact of reducing new intramammary infections during the dry period (treatments, production losses, early culling) would provide additional and show very likely a higher level of return of investment.

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1099 - Understanding dairy smallholders' intention to participate in the control and eradication of bovine brucellosis

Author: Carlos Galdino Martínez-García , José Alberto Albiter-Albiter, Adolfo Armando Rayas-Amor

Objectives
Brucellosis (*Brucella abortus*) is a contagious disease that affects cattle and has a significant economic impact on the livelihood of farmers in developing countries. The aim of the present research was to understand the dairy smallholders' intention to participate in the national campaign to control and eradicate bovine brucellosis in central Mexico.
Material and methods
The study was conducted with 196 farmers who were already engaged in the campaign. The reasoned action approach (RAA) was used as a theoretical framework, and Spearman rank-order correlations were used to analyze the data.
Results
The results showed that farmers had a strong intention to participate in the campaign in the next 12 months. Intention was associated with farmers' years of experience as milk producers and perception of the importance and usefulness of the campaign in addition to farm characteristics. Farmers' intention was also related with positive beliefs (drivers) such as participation in the campaign would prevent the disease in the herd, enable access to governmental support and subsidies, and avoid the spread of the disease to people. Other social referents such as veterinarians, other farmers, and farmers' sons also played an important role in farmers' intention. Although farmers indicated that participation in the campaign in the next 12 months would be easy, a couple of major constraints were identified: the lack of financial resources and communication between farmers and personnel in charge of the campaign.
Conclusions
In conclusion, the three components of the RAA (attitude, perceived norms, and perceived behavioral control) were found to be strong predictors of farmers' intention to participate in the brucellosis eradication campaign and enabled the factors influencing farmers' intentions to be explored in greater depth.

Sustainability and environmental impact

1211 - Reduced methane eructation in smallholder large ruminants with a dietary tannins and citral extract

Author: Peter Windsor Andrew, Julian Hill

Objectives
Large ruminant production is associated with high greenhouse gas emissions (GHGe), with some of the highest emmssions intensity (EI) occurring in several southeast Asia countries. In Laos, EI from cattle is estimated to be 102.9 CO2eq/kg meat produced, compared with average global meat emissions intensity of 33 CO2eq/kg. As recent studies in Laos identified 'emissions control' molasses blocks (ECBs) achieved abatement of 470kg CO2eq/20kg block consumed, a study was conducted with a supplement mix with reported methane reducing properties, containing plant tannins and citral extract, salt, molasses and water.
Material and methods
Groups of housed dairy buffalo (n=7) and pasture-fed grazing beef cows (n=11) were randomly selected, body condition scores estimated (BCS:1-5) and baseline nasa-oral methane eructation measured daily for 2 weeks, using a hand-held methane monitor. All animals were offered 300-400g/day of the mix for a month, with buffalo accessing an abundance of fresh cut forages, whereas the grazing cows had access to rice straw and pasture declining in quality and quantity due to severe drought.
Results
The buffalo consumed all of the mix and retained their BCS of 3, whereas the cattle consumed between 200-300g/day of the mix and their BCS declined from 1.5 to 1.0 during the trial. Analysis of data points (buffalo n=309; cattle n=378) found average methane concentration (AMP) from eructation per animal of both cohorts increased over the 2 week baseline period, then declined during the supplementation period by 36% in the buffalo and 18% in cattle cohorts.
Conclusions
Dietary supplementation with a plant tannins and citral extract reduced AMP from rumen fermentation by up to 36%, confirming previous observations that impressive abatement of GHGe is achievable in large ruminant smallholder production in a developing country. These and other nutritional management innovations including the feeding of ECBs, suggests that improved global food production system efficiencies are achievable, assisting the change management urgently required to reduce methane eructation from large ruminants, and assist efforts to address global food insecurity, plus one health, ecosystem health and climate crisis concerns.

1260 - Scenario analysis of the consequences of heat stress on enteric methane emission in dairy herds

Author: Long Chen , Vivi Mørkøre Thorup, Anne Braad Kudahl, Søren Østergaard

Objectives

Heat stress affects dairy production by decreasing milk production and reproductive performance, and by increasing the risk of diseases and mortality. Although such changes may increase enteric methane (CH_4) emission and decrease animal welfare in dairy herds, they have not yet been thoroughly investigated. This study aimed to quantify enteric CH_4 emission in dairy herds given different levels of severity and exposure to heat stress.

Material and methods

A stochastic, dynamic, and mechanistic herd simulation model was used to simulate changes in milk production and reproductive performance occurring in a Danish dairy herd of 200 Danish Holstein cows producing 11,000 kg of energy-corrected milk (ECM) per cow year. A total of 10 scenarios were simulated by combining 3 levels of severity due to heat stress (low, average, and high), 2 types of effect combinations (milk, and milk plus reproduction), and 3 levels of exposure (one month, 2 months, and 4 months). The effects of heat stress on milk yield, feed efficiency, and reproductive performance were estimated by literature review. For each input parameter, a range of effect sizes was formulated based on multiple studies, and the minimum, average, and maximum of the range were calculated as different levels of severity. These effects were then simulated 500 times over 10 years. The enteric CH_4 production was calculated using the IPCC Tier 2 model based on Scandinavian studies. The CH_4 intensity was calculated as the total CH_4 produced from the entire herd divided by the total ECM yield.

Results

Our preliminary results showed that heat stress, when simulated to impact only milk production, increased enteric CH_4 intensity (g CH_4 / Kg ECM) by 0.8, 1.6, and 3.3 %, when simulating an exposure period of 1, 2, and 4 months, respectively. Simulating the effects from both decreased milk production and reproductive performance increased CH_4 intensity by 0.8, 1.5, and 2.8 %, for an exposure period of 1, 2, and 4 months, respectively. Reduced reproductive performance further led to fewer surplus pregnant heifers in the herd (the number decreased from 14.4 in the baseline herd to 11.5, 8.8, and 2.9 for exposure ranging from 1 to 4 months). Accounting for this, we would expect an even higher increase in enteric methane emission. Sensitivity analysis showed that heat stress at its extreme impact on both milk production and reproductive performance can cause enteric CH_4 intensity to increase by 2.8 %, meanwhile decreasing surplus pregnant heifers to 6, given an exposure of 2 months.

Conclusions

In general, our study revealed additional detrimental effects of heat stress on enteric CH4 emission and emphasized the need for heat stress abatement in dairy production as a tool to mitigate climate change. The increase in CH_4 emission was associated with both the duration of exposure and the severity of heat stress. Our preliminary study did not consider the effect of heat stress on health and mortality which may underestimate the increase of CH_4 emission. Further scenarios including such effects may allow for better estimation of heat stress on CH_4 emission.

1299 - Unveiling the Evolution of Dairy Cattle Farming in Chile over two decades

Author: Paula Toro Mujica

Objectives

This research aims to scrutinize the demographic and structural transformations within the dairy cattle farming sector in Chile.

Material and methods

Utilizing micro databases from the Agricultural Censuses of 1997, 2007, and 2021, the study focused on farms housing at least one dairy or dual-purpose breed cow. Variable selection was facilitated by using the Microsoft Access® program to create "Query Tables", leading to the development of databases for each study year and a subsequent amalgamation into a comprehensive global database.

The global database featured key variables for each farm, including census year, geographical region, cattle numbers by category, number of other livestock species, cultivated area for crops, forage, natural and improved grasslands, orchard trees, and forest species. System intensification and specialization variables such as stocking rate, surface allocation for each grassland and crop type, and the significance of cows within the cattle and livestock categories were calculated from the database.

Statistical analyses, including ANOVA tests and mean comparisons, were conducted to assess the impact of the census year on the variables under study.

Results

Results indicate a significant reduction in the number of farms housing dairy cows over the past 24 years, decreasing from 46,900 in 1997 to 14,222 in 2021. This decline corresponded to a decrease in the national herd from 761,835 cows in 1997 to 492,760 in 2021. The Metropolitan region experienced a 90.6 % decrease in farm numbers, a value much larger than that of the country as a whole. In 1997, 88.7% of farms were situated in the south-central and southern regions, increasing to 94.4% in 2021.

At the national level, the cattle and total stocking rate increased by 18.8% and 12.5%, reaching 1.14 and 1.55 animal units (AU) per hectare respectively, in 2021 ($p<0.01$). The average farm herd size rose from 27.9 AU in 1997 to 50 AU in 2021. Furthermore, the percentage of cows AU increased from 59.1% in 1997 to 66.0% in 2021 ($p<0.01$).

Regarding forage utilization, differences in the percentage of surface area occupied by natural, improved, and planted grasslands were observed between the evaluated years ($p<0.01$). However, no distinct trend was discerned over time, with the percentage of natural grassland decreasing from 33.1% in 2007 to 30.4% and subsequently rising to 35.4% in 2021.

Conclusions

These findings signify a notable decrease in dairy cow production farms in Chile, and a large relocation of dairy farms from the Central and Metropolitan region to South-Central and Southern regions possibly driven by land prices and climate changes. Furthermore, the data show a concurrent intensification of production systems, evidenced by an elevated cattle stocking rate and increased specialization in the activity, particularly concerning the percentage of AU corresponding to cows. Factors contributing to these shifts include competition with other profitable agricultural activities, notably fruit growing and viticulture, the impact of climate change on forage production, and an increase in residential plots in suburban and rural areas. Given the country's widely diverse North to South climates, it is imperative to analyze regional-level changes and their underlying causes, as well as changes in production efficiency and environmental impacts.

1553 - The Use of Machine Learning to Predict Cow and Herd Level Outcomes and Optimise Dairy Herd Management

Author: Professor Andrew Bradley, Luke O'Grady, Martin Green

Objectives

Modern dairy herds have access to increasing amounts of information about their cows, their productivity and health status, but the ability to process, optimise and maximise the value of these data is often compromised by a lack of time, necessary skills and appropriate software platforms. Given the impact of agriculture in the context of climate change, there is also an increasing need for producers and their advisors to be able to make evidence-based decisions about optimal approaches to reducing environmental impact of their enterprises. The aim of the research outlined in this abstract was to use the latest machine learning techniques to develop cow and herd level predictive models which could then be applied in automated systems to optimise decision making in dairy production systems from the perspective of productivity, animal health and welfare and climate impact.

Material and methods

Data was available from over 200 UK dairy herds with individual cow records covering the period from 1975 to 2023 for in excess of 500,000 adult cow lifetimes. Data was collated and cleansed and animal 'states' determined using the TotalVet© herd health analysis software prior to anonymization and export in a csv format suitable for further analysis using "Python" and "R". A variety of different existing and novel approaches to data interpretation and analysis have been adopted to develop models covering all areas of dairy herd health from fertility to udder health, lameness and Johne's disease and milk yield. These have been used alone and in combination to develop individual cow prediction models and disease pattern tools to inform on farm interventions as wells as a 'whole farm' model facilitating modelling and prediction of dairy herd production and carbon footprint.

Results

To date, disease pattern tools have been developed for Mastitis and Lameness, with the former now fully automated and available to UK dairy farmers (https://ahdb.org.uk/knowledge-library/mastitis-pattern-analysis-tool). A similar pattern tool for Johne's disease based on a novel analysis of Johne's Disease antibody screening is nearing completion and will be launched in early 2024.

In addition, tools to predict treatment outcome of clinical and sub-clinical mastitis, the probability of development of a new intramammary infection, the likelihood of conception and lameness, and the probability of infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) have also been developed. These can be used as 'stand-alone' tools, but have also been, or are being combined into a 'whole farm' model (REMEDY - https://cloud.remedy.farm/) which allows users to model different scenarios and their impacts on future productivity, health and environmental impact.

Conclusions

The wealth of data available on modern dairy farms combined with the latest data analysis and machine learning techniques offers a step change in our ability to improve and optimise health and productivity on dairy farms. Automation and the ability to tailor such approaches to data management to individual farms offers a unprecedented opportunity for farmers and their advisors to improve efficiency, meet consumer expectations and prepare for the challenges posed by climate change.

1704 - Influence of reduced age at slaughter on the carbon footprint of beef herd under the life cycle assessment metric

Author: Lais Abreu, Vanessa de Paula, Bruno Carvalho, Alexandre Souza, Sofia Albertini, Emanuele Gricio, Ligia Rebeis, Fernando Mori, Pietro Baruselli

Objectives

The growing demand for sustainable development has led to the adoption of practices and technologies in agriculture and livestock production that aim to reduce greenhouse gas (GHG) emissions. The Life Cycle Assessment (LCA) is a tool used to analyze the environmental impacts of products, processes, or services throughout their entire life cycle. It is used to support the development of technologies and identify mitigation solutions. LCA studies on beef production systems in the tropics revealed that CH4 emissions are influenced by herd diet, genetics, and fertility. Therefore, the age at which animals are slaughtered (RAS) can affect the herd's composition and performance. The aim of this study was to investigate how reducing RAS affects CH4 emissions by performing a sensitivity analysis of the LCA of beef production

Material and methods

A comparison was made between the production and CO2 equivalent per unit of beef (corrected for kilogram of live weight content) of cows and steers through hypothetical scenarios. The scenarios included animals with an average RAS of 24 months (identified as 24-RAS) and animals with an average RAS of 36 months (identified as 36-RAS). Data was collected from a full-cycle commercial farm in Paraná, Brazil that raises Nelore and crossbred beef cattle, producing an average of 1,369,466 kg per year. According to a study that followed ISO 14040 and ISO 14044 requirements, the carbon footprint of beef production was estimated using the Open LCA 3.11.1 software. The software was used to model and estimate the amount of CO2 equivalent emissions (CO2 eq.) produced by beef production. The cradle-to-farm-gate frontier includes animal management, natural resource use, energy, inputs, waste, and direct and indirect emissions. The study identified the potential environmental impacts that are associated with the entire life cycle of meat production. These impacts were analyzed according to their level of importance, and the following factors were identified in order of significance: enteric methane, waste management, food production within the farm, food production outside the farm, transport, and energy, and others.

Results

The RAS duration of 24 months resulted in a total of 15,6 CO2 eq./kg of beef GHG emissions. For the 36-month RAS group, the amount of CO2 emissions per kg of beef produced was 17.7, which represents a 13.5% increase. It was found that the largest proportion of the carbon footprint of emissions from the operating system was due to the emission of enteric methane [24-RAS= 11.3 vs. 36-RAS= 13.1]. It was observed that the other variables evaluated were also lower for the 24-RAS group (Waste management: 24-RAS= 2,05 vs. 36-RAS= 2,14; food production on farm: 24-RAS= 0,26 vs. 36-RAS= 0,29; food production outside off farm: 24-RAS= 1,92 vs. 36-RAS= 2,10; transport and energy: 24-RAS= 0,010 vs. 36-RAS= 0,012; and others: 24-RAS= 0,020 vs. 36-RAS= 0,023).

Conclusions

It has been concluded that reducing RAS levels is essential to increase productive efficiency and decrease CO2 emissions per kilogram of beef produced.

Short communications

1557 - What is the effect of consuming dairy products and beef on the climate?

Author: Frank van Eerdenburg

Objectives
To determine the effect of consumption of dairy products and beef on the climate
Material and methods
literature review
Results
In newspapers and documentaries about climate change, there are two dominant messages: stop using fossil fuels and stop eating meat and dairy products. I fully agree with the first message. The second one, however, is not right and needs some further explanation. Fossil fuels are Carbon (C) that is released into the atmosphere when burned to CO_2. The result of this is that the concentration of CO_2 in the atmosphere increases and this has an effect on the climate. We call, therefore, CO_2 a greenhouse gas (GHG). Other gasses have a similar effect (e.g. CH_4 and N_2O). CO_2 is absorbed by plants, like grass, and split into C and O_2 by a process called photosynthesis. The C is used by the plant to grow and thus 'stored' in the plant. In this way plants reduce the amount of CO_2 in the air. Cows eat grass, a plant that humans cannot digest properly. In this way they ingest the C that is stored in the plant. They metabolize the C into CO_2, which is exhaled, and thus 'recycle' the C back into the air. This can be C that was stored in the grass just a few hours or days before. This is called 'the short cycle' as opposed to 'the long cycle' of the fossil fuels. The C in the feed is not only converted into CO_2, however, but also in CH_4, a far more potent GHG, which is also exhaled and thus released in the atmosphere. The lifetime of CH_4 is quite long (up to12 years) but recent reports (IPCC AR6, 2022) mention that this has been overestimated by a factor 3-4 in previous reports and calculations. So in fact, CH_4 is not a bad as was assumed. Bearing this in mind, CH_4 is still an GHG emitted by cattle and every option for reduction is worth investigating. For example, CH_4 emissions can be substantially (30-40%) reduced with pasture management (Carmona Flores et al. 2020), or feed additives (Kebreab et al. 2022) However, reducing the number of animals in areas with an intensive, highly developed, cattle-industry, like the Netherlands, is not the way to achieve this. If we reduce herds in developed regions, we'd be reducing the most efficient animals in the world. The ones that produce more food with less environmental impact. Neither does it make sense to cut back on animal agriculture in developing regions, where population is growing faster, and where nutrition and livelihoods are at stake. This brings another point to our attention: How do we produce food for the people in the world? The amount of arable land is limited and a large part is only good to grow grass. For example, On the 800,000 km^2 of the Pampas in South America there only grows grass. Of the land, in use for agriculture, in the Netherlands 30% is to wet to grow anything else than grass. If we would not have these lands grazed by ruminants, they would produce nothing edible by humans. Cows are thus very helpful in feeding the world population. Furthermore, if the grass is not eaten by animals it will be not eaten at all and thus die and rot. It is converted into CO_2 and CH_4 as well and thus there will be no benefit for the climate. Especially in wet lands the amount of CH_4 ('swamp-gas') produced is large.
Conclusions
So the consumption of dairy products and meat from grass-fed cattle is not detrimental for the climate at all. Slowing the growth of the human population and increasing the number of trees

(to capture CO_2 from the air) is far more effective in reversing the climate change than reducing the consumption of dairy products and beef, from grass-fed cattle.

References:

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1034 - Ultrasound made simple: How to diagnose abdominal and thoracic diseases with a transrectal probe. The BovATUS (Bovine Abdominal Thoracical ultrasound) method

Author: Camilo Cabra Garcia

Objectives

Ultrasound has become a powerful and essential tool in reproduction diagnosis for bovine practitioners, but its use in other types of diseases on-field is scarce. This imaging technique is among the complementary examinations (laboratory and tissue sampling), one of the quickest and easiest to use even in extreme field conditions when providing an accurate animal health status. Nevertheless, it is usually believed that the transrectal linear probe is not convenient for using on other parts of the body. Consequently, and due to the lack of training and the absence of a given method for using this probe in thorax and abdomen, proper diagnosis, treatment and prognosis of related pathologies could be stalled.

Therefore, the objective is to propose an on-field short protocol (7-10 minutes) to fully examine the abdomen and thorax of adult cows with a transrectal probe of a regular ultrasound machine used for daily reproductive diagnosis. This is achieved by modifying some machine configurations to observe the most important organs in these regions.

Material and methods

An examination protocol was created for one of the most popular ultrasound machines ([IMV Easi Scan GO], 5-7.5 MHz transrectal linear probe) used by field veterinarians in the world. Isopropyl alcohol (70%) was used as a coupling medium between the probe and the skin to avoid clipping the animal's hair, save time and get an image quality comparable to that which can be obtained by shanving the skin and using ultrasound gel. Two settings should be modified to perform the exam: depth and gain. Before starting the exam, you should increase the depth of penetration to the maximum (12cm) available in order to see the deepest structures. Because the gain must be increased when scanning the liver (right abdomen) and this is the only one of all the organs that requires this modification it will be left for the end of the exam. It is suggested to start on the left side of the animal to modify the gain only once. Pneumonia is a highly prevalent pathology (sometimes asymptomatic) and for this reason it is recommended to begin with the left thorax, scanning the left lung (fifth to twelfth intercostal space). The heart will be then observed in the mid-ventral zone (fourth to fifth intercostal space). After this, the scan continues to look for the wall of the reticulum and the cranio-dorsal sac of the rumen (watch carefully the biphasic movements). These two organs can be found from ventral to dorsal up to the level of the elbow; they form a triangular region where signs of inflammation and infection could be observed. The rest of the rumen occupies the left caudal abdomen. When the left side observation is terminated, the operator moves to the right side and proceed with right thorax (lung). Then, moving to the right caudal abdomen the small intestine (motility, content, wall thickness and loops diameter), large intestine, and the mesentery (presence of edema and lymph nodes) are scanned. Next, the pyloric part of the abomasum (half-moon shape) and the abomasum (content, folds and the wall) are observed. Once this is finished, the gain needs to be increased enough to see the liver without losing the details of the tissue (fifth to last intercostal space) and the right kidney. In the liver, you will see the parenchyma (homogeneous echogenicity) and the hepatic and portal vessels. Due to its position (deep in the abdomen), with the linear probe, it will be almost impossible to see the caudal vena cava in an adult cow

and some portions of the liver (medial and diaphragmatic). When scanning cows with body condition greater than 3.75 (score 1-5), due to fatty tissue (reflecting the ultrasound), observation of the organs will be difficult.

Results

With this proposed method, lesions such as reticuloperitonitis, right and left displacement of abomasum, liver abscesses and calculi, ascites, ileus, right renal cysts and lithiasis, abnormalities of the gallbladder, pulmonary consolidations, hydrothorax, enlargement of the mesenteric lymph nodes, peritonitis, valvular endocarditis, pericardial effusion, among others can be diagnosed or suspected.

Conclusions

Quick, efficient and precise ultrasound examination can be performed by using the BovATUS method, refining treatment and prognosis (decision-making) at the individual and herd level, and improving general animal health status, welfare, and finances of the farm.

1613 - Clinical and cytological evaluation of the cervix in relation to the estrous cycle in grazing dairy cows from high-altitude tropical herds in a pasture-based systems

Author: Andres Timarán Rivera, Dario Antonio Vallejo Timarán, Nelly Porras Diaz

Objectives

The objective of this study was to quantify the clinical, endoscopy and cytological evaluation of the cervix in postpartum dairy cows from high-altitude tropical dairy herds in a pasture-based systems and, to evaluate the concordance of the cervix diagnosis with the estrous cycle.

Material and methods

A prospective cross-sectional study was designed in postpartum dairy cows from a herd specialized in pasture-based milk production in the high tropics in region of Nariño, Colombia. The sample size was calculated to test a null hypothesis of equality in the diagnosis of clinical cervicitis with the conventional method in comparison to the diagnosis of clinical cervicitis associated with the time of the estrous cycle. Sample size was calculated in 82 animals to detecting a significant difference (95% CI - 80% Power) for cervicitis diagnosis between vaginoscopy method and vaginoscopy + estrous cycle method. Cows were included in the study based on the following criteria: dairy breed (e.g. Holstein), non-interventional calving by caesarean section, no clinical signs of systemic disease (e.g. depressed attitude, anorexia, fever, downer cow) that required the use of antibiotics and anti-inflammatories. Information about breed, parity, occurrence of retained placenta, body condition score was collected. Additional data on grazing, and transition period feeding were also collected. In the cows included in the study was performed sequentially: a) reproductive evaluation through transrectal palpation and ultrasound, determining the status of the uterus and ovaries; b) vaginoscopy for the evaluation of the cervix as follows: Grade 0 [No prolapse, no congestion] - Grade 1 [Redness or congestion of the tissue] - Grade 2 [Redness + Prolapse of the second cervical ring]; c) video-endoscopy of the cervix determining macroscopic alterations based on the following criteria: changes in tissue color; tissue integrity; presence of injuries; injury severity; cervical ring prolapse; opening of the cervix; presence and visual characteristics of secretions; d) cytological examination of the cervix and endometrium. Between 60 and 70 days postpartum, cows were enrolled in an ovulation synchronization program and inseminated at a fixed time. Pregnancy diagnosis was performed 30 ± 2 days after breeding. The data were analyzed using descriptive statistics to determine the frequency of clinical, endoscopic, and cytological changes of the cervix. Subsequently, using the maximum likelihood ratio test (Likelihood-ratio) the agreement between the cervix evaluation findings and the phases of the estrous cycle were determined. Differences in the diagnosis of cervicitis by vaginoscopy method and vaginoscopy + estrous cycle method according pregnancy rates after artificial insemination were also stablished. Some diagnostic guidelines for cervical inflammation were determined taking into consideration the findings on video endoscopy and the phase of the estrous cycle.

Results

Preliminary results shown that clinical cervicitis (Redness + Prolapse of the second cervical ring) were diagnosed in the 30.4% of the cows. The 47.1% of the cows with clinical cervicitis diagnosis were also defined as estrous cow. In the same way, the 35.3% of the cows with clinical cervicitis diagnosis were also defined as estrous cows, had normal cytological examination (at cervical and endometrial level) and get pregnant after first artificial insemination. Strong concordance

between vaginoscopy and estrus cycle were found. Some additional changes found in cervix endoscopy were related with inflammatory uterine conditions.

Conclusions

According to preliminary results, changes in grade 2 cervix of cows in estrus in dairy herds in the high tropics may be related to changes associated with heat and not with inflammatory changes. The use of endoscopy and vaginoscopy considering the time of the estrous cycle can increase the reliability of the diagnosis of clinical cervicitis, favoring the implementation of therapeutic protocols, prevention measures and improving fertility.

1721 - Macro-minerals elements behavior in cows transition period

Author: René Rosiles Martínez, Ulises Jesús Bautista Pérez, Rodrigo González López, Arturo Federico Olguin y Bernal, Alejandra Vargas Vera, Ángel Daniel Torres Ruíz

Objectives

Cows blood serum macro-minerals elements (ME= P, Na, K, Ca and Mg) concentration pre and postpartum (transition period) should be balanced in feed according to milk production.

Transition period, in cows is a switching from dry state to milk production and is considered as a critical period. At this time, cows by themselves tries to control (reestablish) these abnormal conditions. Either to an increase of feed intake, or to absorption from tissues storage or metabolic active (blood and bones) found in the postpartum period. It is also considered to increase energy and mineral demand due to the increasing milk production. Usually, cows are unable to counteract this situation, so clinically are observed as sick animals.

Blood serum minerals elements identification in caws transition period is needed in order to diet supplement them before caws are sick.

Actually calcium is one of the ME that requires more attention due to its typical rapid postpartum depletion, But P, Na, K and Mg, are not considered in a daily control. Since Ca is the only element considered for the downer syndrome, It is not known if serum quantity modifications of P, Mg, Na and K plays any function in this Downer cows syndrome or predispose to other diseases such as abomasum displacement, placental retention, decreased fertility, among others.

Other ME such as: phosphorus, potassium, sodium and magnesium changes in blood serum concentrations during the transition period, is needed to know them because they are secreted in milk throughout lactation. ME variation in concentrations before or after parturition, even throughout lactation, will be useful., to know its variation. However, it is important to regulate them from external source, because, excess o deficiency may cause a disease. ME Interaction is complex, for example, hyponatremia or hyperkalemia decrease the absorption of Mg in the rumen, while a condition of hyperphosphatemia predisposes indirectly to hypocalcemia. Hypomagnesemia is one of the main factors in hypocalcemia, with hypomagnesemia; nervous system the kidney and bone are less responsive to parathormone, so Mg inclusion in the diet is needed as complement.

Material and methods

Atomic absorption spectrometry was used to monitor blood serum calcium, magnesium, sodium, potassium and phosphorus levels, in 12 cows, during transition period. (pre and post parturition).

Results

Cows blood serum mean mineral elements (ME. µg/ml). content, pre and pos-parturition.

	µg/mL pre parturition	post parturition	Situation
Ca	12.05	9.48	hipo
P	9.5	18.69	hiper
Na	527.05	526.4	hipo
K	27.95	25.43	hipo
Mg	2.4	1.9	hipo

Ca, Mg, and K had a typical decrease as would be expected in postpartum cows, unlike it has not been reported, what has been reported with P, which in this case was found to have an increase in its concentration in the postpartum period, and Na which remained similar. There were cows in which mineral behavior was contrary to the average, this is due to the fact that variations between ME have been identified in the organism with the purpose of maintaining an electrolytic balance and the blood pH, as well as to maintain cellular functioning, and their interaction is also carried out with ME.

Conclusions

Postpartum period is critical for the life of the cow, where the downer cow syndrome is a reason to determine culling. There are different causes for its presentation, among them; ME alterations such: hypocalcemia, hyperphosphatemia hyperkalemia, hypernatremia and hypomagnesemia. However, it should be taken into account that other ME can be diminished and present the same clinical signs. Therefore, diagnosis and therapeutic plan must be carried out quickly to reestablish ME, as well as energy deficient condition.

1522 - The use of a topical, rapid-drying liquid barrier dressing containing copper acetate and zinc chloride as a treatment for Udder Cleft Dermatitis lesions in dairy cattle in the UK.

Author: Mike Kerby

Objectives

To investigate the effect of a rapid-drying liquid barrier dressing containing copper acetate and zinc chloride applied once a week to Udder Cleft Dermatitis lesions .

Background :

UCD (Udder Cleft Dermatitis) is becoming an increasingly recognised skin problem in dairy cows affecting either the fore-udder attachment with the abdominal wall and/or the skin between the two udder halves.

The lesions vary from mild erythema, papules, pustules and crusts through to large areas of necrotic and malodorous tissue with purulent exudate.

UCD may increase the risk of clinical mastitis and embolic pneumonia as well as presenting as haemorrhage from subcutaneous veins.

The precise aetiology of UCD has not been elucidated but has been associated with mange mites , treponemes and bacterial colonisation .

UCD lesions heal poorly. The size of the wound has an effect on the likelihood of improvement- it is therefore important to detect and treat lesions early, though these are often poorly recognised by herdspeople and their importance not recognised.

Scientific research into treatment for UCD in dairy cows is limited.There is no current licensed treatment for UCD in the UK

Material and methods

Animal Inclusion criteria: Animals with active UCD lesions grades 2 to 5 (Ekman *et al.,* 2021).

Treatment and Data Collection: Animals were treated once a week with the liquid barrier dressing for a minimum of 4 successive weeks by a veterinarian. The lesion was cleaned with a 7.5% w/w Povidone-Iodine solution , dried with a paper towel , photographed, assessed for lesion grade, malodour, wound exudate, lesion depth and signs of healing (scab formation, granulation tissue bed, re-epithelisation).

An independent veterinarian looked blindly at the photographs of each case sequentially and assessed whether the lesion was better, the same or worse than at the previous visit (“lesion/ healing progression “).

Results

A total of 26 cows on 3 farms (2 in the South-west and 1 in the East of England) were recruited, with 4 x weekly applications on Farm A (n=8), 5 x weekly on Farm B (n=5) and 9 x weekly on Farm C (n=13).

For the 26 animals , after 4 weekly applications ,there was a 62% reduction in the number with malodour (81% at Week 0 ; 19% at Week 4), a 50 % reduction with exudate (77% down to 27%), a 70 % reduction with deep involvement of the subcutis (77% to 7%) and a 50% increase in the number showing re-epithelialisation (46% up to 96%).

For the 13 cows on Farm C (Grades 2 to 4 at Week 0) that had 9 x weekly treatments , 10 (77%) were fully healed by Week 9 and the other 3 (23%) were showing full signs of re-epithelialisation.

Of the 104 weekly follow ups, 77% were judged as improved , 20% showed no change and 3 % were worse .

Overall , there were improvements in the grade of each UCD lesion after 4 x weekly treatments . The percentage of cows with grade 1 (normal) was 0% at Week 0 and 9% at Week

4 , grade 2 : 11.5% and 39%, grade 3 :46% and 43% , grade 4 : 31% and 9% and grade 5 : 11.5% and 0%.

Conclusions

Once a week application of a liquid barrier dressing containing copper acetate and zinc chloride appeared to promote healing of UCD lesions in dairy cattle with marked reductions in malodour, exudate and depth of the lesion as well as increased signs of re-epithelialisation.

The clinical impression between Farms A+B and Farm C is that a more prolonged course of application may be required to achieve full healing of grade 4 and 5 lesions (or a more frequent application - perhaps until the grade of lesion has decreased to 3 or less).

Thus, further studies are on-going looking at applications on Days 0, 2 and 7 followed then weekly until Day 42 or longer. A full set of data is anticipated in Spring 2024.

Reference.

Ekman L., Nyman A.K., Persson Waller K. Recovery from udder cleft dermatitis in dairy cows .*J Dairy Sci* 2021 104 3532-3546

1187 - Initial report: Osteochondrosis dissecans emerging as important lesion in postmortem exams in Mexico.

Author: Rafael Ramírez, Carlos Corona, Esteban Gallegos, Horacio Herrera, Sergio Terrazas, Eduardo Velasco, Cecilia Ramírez

Objectives

OBJECTIVE: Osteochondrosis (also osteochondritis) dissecans has become an important condition in feedlot cattle in Mexico. The aim of this report is the presentation of several cases with severe articular lesions of osteochondrosis (osteochondritis) dissecans (OD). The lesions were incidentally recognized in necropsies of commercial fattened animals during the last two years in feedlots in Mexico.

Material and methods

MATERIALS AND METHODS: This report describes the gross morpho pathological diagnosis and the proposed pathogenesis of OD lesions in routine necropsy examinations of dead animals with different other diagnosis during 2022 and 2023. The material revised was obtained from seven large feedlots in Mexico (more than 45,000 heads in the smaller one and above 150,000 in the largest). These feedlots are located at the North gulf zone (3) the Midwest (1), Northwest (2), and North (1 feedlot). The study included commercial bovine breeds with zebu crossbreed, mostly gathered from southeast Mexico. Weights range from 220-380 Kg at receiving and finished around 580 Kg for market. Most cases corresponded to deaths during the mid-term (60 to 90 days in feed) and long-term period (90 and over days in feed). Few cases corresponded to fattened animals close to slaughterhouse weight. None of the feedlots were located over 250 m AMSL and their climate ranged from humid tropic to desertic zones. All these feedlots but one exception have their own slaughterhouse and move the fattened animals by driving the entire finished pen to slaughterhouse (≈ 3 K).

Results

RESULTS AND DISCUSSION: From 80 necropsies reviewed most of the dead animals (78%) had articular lesions characteristics of OD; although as mentioned, postmortem diagnoses were other different such as bronchopneumonia, ruminal acidosis, pericarditis, and heart failure. The OD lesions were incidental findings in the radio-carpal and tibio-tarsal joints, routinely examined. Nonetheless, some of these animals were affected and prostrated previously, indeed, when these were forced to move, they moved supported over their radio-carpal joints. Few cases of finished animals (2%) with histories of fallen while the horsemen drive the entire pen to slaughterhouse (3 km distance) were also included. Other cases that were identified clinically with lameness but at necropsy showed laminitis, pododermatitis or arthritis (8%) were discarded. In some (12%) of the necropsied animals, no lesions of OD were identified. The animals with lesions attributed to OD were the higher proportion but not the cause of death. The lesion is characteristic grossly. The review of joints in a postmortem study is routinely observed and no previous sight of high prevalence of OD lesions had been registered before. During past two years and this year, a severe long lasting drought season have occurred in Mexico and these conditions impacted in the condition of gathered cattle in the southeast Mexico; this region is the main provider (≈75% of inventory) for feedlots in Mexico. The nutritional status in these animals is normally deficient. Recent cases of enzootic vesical hematuria (interpreted as overgrazed consequence) and hyperplasia/metaplasia of parotid gland ducts epithelium (compatible with Vit. A deficiency) are indicators of deficient nutrition in origin not entirely compensated during fattening. In the case of OD there is a consensus about its multifactorial condition, including over nutrition/rapid growth in confined animals, nutritional deficiencies, and a genetic predisposition. Excluding the latter one the formers could be related with the presentation of the OD in the feedlot cattle in Mexico. More studies are required for better characterize this syndrome in Mexico and worldwide in cattle.

Conclusions

CONCLUSION: This study demonstrated that OD is a serious condition frequently observed in feedlot cattle in Mexico. This lesion should not become some common.

1062 - Randomized, non-inferiority trial evaluating the efficacy of a novel teat sealant in pasture grazed dairy cows

Author: P. Mehrtens, E. L. Cuttance, W. A. Mason, J. M. Swinkels, R. Nortje

Objectives
This non-inferiority field study was designed to compare the efficacy of a new (IVP) internal teat sealant (ITS) with the NZ market-leading ITS (CPT), in the New Zealand (NZ) market. The objective of the study was to demonstrate non-inferiority of the IVP to the CPT in a NZ commercial dairy setting by comparison of i) risk of cow-level clinical mastitis from dry-off to the first 30 days post-calving between IVP and CPT treated cows, using a non-inferiority margin of 2%, ii) comparison of the presence or absence of ITS in quarters at calving between IVP and CPT treated cows, as reported by farmers, using a non-inferiority margin = 5%, and iii) comparison of cow-level somatic cell counts (SCC) at the first herd-test post-calving between IVP and CPT treated cows by setting the non-inferiority margin at 10,000 cells/mL.
Material and methods
Cows from two commercial NZ dairy farms were enrolled in the study based on the following selection criteria: planned dry period between 60 and 120 days, at least three functional quarters, body condition score > 3.0 (Dairy NZ BCS), lameness score of 0 to 1 (Dairy NZ lameness scoring system), individual cow SCC < 150,000 for mixed-age cows, or SCC<120,000 if a first lactation heifer, at the most recent herd test (<80 days prior to dry off2, no clinical mastitis in the current lactation (including on the day of dry-off), and acceptable teat end damage (Teat end score of N or S), positive pregnancy scan within 30 days of dry-off. Enrolled cows were randomly assigned to IVP (ShutOut® (MSD Animal Health), or CPT positive control (Teatseal®, Zoetis) treatment groups. Treatments were administered at the cow-level at dry-off, with retention analysed at the quarter-level at first milking, and the other two outcomes (see objectives) analysed at the cow level. The veterinarians and technicians administering the product were not blinded when administering treatment products; however, all farm staff involved in assessment of various parameters were blinded to the treatments. The biometrician was also blinded to the treatment groups, while analysing the data.
Results
A total of 1105 cows were randomly enrolled across the two farms, 555 in the IVP group and 550 in the CPT group. (i) IVP was non-inferior to CPT for the incidence of clinical mastitis. The risk of mastitis was 0.7% lower (95% confidence interval = 3.1% lower to 1.6% greater) in cows in the IVP group compared to CPT. In total, 4.1% (44/1081) of cows had a case of mastitis between dry-off and 30 days post-calving. There were 3.7% (20/540) of cows in the IVP group with clinical mastitis compared to 4.4% (24/541) of cows in the CPT group with clinical mastitis. (ii) IVP was non-inferior to CPT for retention. Sealant was retained in 79.6% of quarters treated with IVP compared to 74.2% of quarters treated with CPT. IVP was also superior to CPT for retention, with quarters treated with IVP associated with a 26% increased odds of retention (95% CI = 2% to 44%; p=0.036). (iii) IVP was Non-inferior to CPT for SCC. The incidence risk of SCC was 14% lower in cows in the IVP group compared to animals in the CPT group (95% CI = 28% lower to 2% greater). The predicted mean SCC was 84,000 cells/ml for cows in the IVP group and 99,000 cells/ml for cows in the CPT group.
Conclusions
Analysis of the data shows that IVP is non-inferior to CPT for the prevention of clinical and sub-clinical mastitis over early lactation under NZ field conditions. Furthermore, in this study, IVP performed better that CPT in terms of retention of product in the teat canal.

1204 - Causes of sudden death in cattle, a practical way to differentiate them in the field

Author: Ulises Jesús Bautista Pérez, Arturo Federico Olguin y Bernal, Diana Patricia Martínez Albarrán, Miguel Ángel Quiroz Martínez Martínez, Rodrigo González López, Jazmín De la Luz Armendáriz, Erika Georgina Hernández Rojas, José Francisco Rivera Benitez, Martín Fernando Hernández Moreno, Mariana Colorado Solís

Objectives

In Mexico it is estimated there are around 75 million of bovines and a little more than 1.1 million of farms. Most of these farms have a purely extensive production approach, where the cattle are grazed day and night and the farmer generally does not practice night surveillance.

Different types of mortality can be manifested with different classifications, being considered acute or chronic, infectious or non-infectious, natural, trauma or accident, or even euthanasia, furthermore, we can add that mortality may include one or several animals in a determined period. In any of the presentations, the death is of economic impact, so the determination of the cause of death and its prevention is of utmost importance.

Sometimes the death of cattle occurs suddenly making it impossible for the farmer to suspect a cause or clinic signs that can be identified, which requires more precision in the inspection of the incident.

Material and methods

When an animal dies, it goes through different stages of decomposition. Early *postmortem* changes include *Algor mortis*, *Rigor mortis* and *Livor mortis*. Late *postmortem* changes include processes of decomposition or putrefaction as emphysematous stage, colicuefaction and skeletal reduction stages.

The decomposition process varies in time depending on the ambient temperature and humidity, accelerating if there are warm conditions and high humidity. Cold environmental conditions can cause delayed decomposition due to slow bacterial growth. Decomposition time is also fast in cattle with septicemic infections or if scavenging is present.

The carcass can be consumed by some types of necrophagous arthropods, as well as by the presence of scavengers. Scavengers commonly found in Mexico are vultures (*Coragyps atratus*) and small wild carnivores that show opportunistic scavenging behavior. Several types of scavengers can be fed on the same carcass, so the integrity can vary.

Results

It is utmost important to check the suroundings where the bovine died. If there is grass around the carcass, it can be observed "burned" due to the physicochemical changes caused by the release of liquids, with resprouting of the grass after several days, this information can be integrated to estimate the time of death.

The number of dead animals and the period in which the deaths occurred also helps to determine the probable cause of death. Several dead animals in a short period of time may suggest a probable intoxication, while several dead animals often several days apart, may indicate the presence of infectious diseases. There are cases of acute death where only one bovine was affected, in this event we can consider an isolated type of intoxication.

There are several common causes of acute death in cattle, including intoxications, anthrax, clostridiasis, attacks by predators and some climatic accidents such as electrofulguration. Several dead animals in a short period of time may suggest food poisoning or contaminated water. Another cause that can involve several animals is lightning strikes. Typically, carcasses are observed under a tree with the same time of decomposition, the tree that received the lightning strike, can show injuries and the death of the tree can be observed.

Ophidian poisonings are also frequent in different regions of the country, and their presence will depend on the type of venomous snakes found. Typically, there may be a greater degree of

decomposition in the region of the body where the bite occurred, commonly in the rostral or ventral regions. In the carcasses, hooves, horns and hair are commonly detached, and together with other cases of intoxication, scavenging by vultures is not usually present, unlike due to infections, trauma or lightning strikes.

Calves are usually affected from attack by canines, felines, or ursids. The pattern of attack and the number of killed animals is important in determine which type of predator was present. While felines and bears commonly attack one or two animals, canines often kill larger numbers. While in wild animals the attack patterns are more specialized, the injuries by dogs have more disordered pattern.

Conclusions

Determining the cause of death of an animal in a state of decomposition can be complex, however, observing the evidence found in the surrounding can bring us closer to a diagnosis. It is essential to have an excellent propedeutic method focusing on clinical history and anamnesis, and preferably, to be assisted by diagnostic laboratories.

1254 - Extensive and severe necrotic tongue lesions in Canadian feedlot cattle

Author: Meghan Brookhart, Katie Waine , Greg Dimmers, Dayna Goldsmith, Eugene Janzen , Vanessa Cowan, Barry Blakley, Felipe Reggeti, Francisco Uzal, Timothy Olchowy, Beverly Morrison, Erin Zachar, Lindsay Rogers

Objectives
During the summer and fall of 2023 over 1,000 cattle in multiple pens in a Canadian feedlot presented with severe tongue lesions suggestive of traumatic injury. This presentation will describe the clinical details of this case, investigations that took place on-farm and the extensive further diagnostic testing.
Material and methods
Examination of the environment, feed, water, cattle behaviour and handling systems was undertaken as well as diagnostic testing including necropsy, histopathology, bacteriology, virology and toxicology. A literature search was also conducted for previous cases and differential diagnoses, and the case was discussed with local, national, and international colleagues and experts.
Results
There was no evidence of sharp objects, rough surfaces, exposed equipment or stray voltage found on examination of the pens, feed troughs, waterers or handling systems to support a traumatic cause. Pathological assessment found a necrotising and suppurative glossitis. Mixed bacteria were found on bacteriology of tongue samples and considered commensals or secondary invaders. Several clostridial species associated with necrotizing myositis were ruled out. There was no evidence to support a viral insult. Multiple feed samples were analysed for mycotoxins and results were unremarkable. Subsequent analysis of hard aggregate masses discovered within the grain pile and feedbunk found a high level of ergot toxins and investigations are ongoing.
Conclusions
This case details an extensive investigation into severe tongue lesions affecting multiple feedlot cattle in a Canadian feedlot.

1618 - The number of white blood cells in European Bison (Bison bonasus) increases in late autumn - a quirk or the rule? Preliminary data.

Author: Professor Romuald Zabielski, Karolina Ferenc, Elwira Plis-Kuprianowicz, Michał Michał Krzysiak, Magdalena Larska, Maria Sady, Professor Zdzisław Gajewski

Objectives

The European bison (*Bison bonasus*) population in Poland is around 2600 individuals living in several independent areas, though of similar climate of lowland Central Europe. The greatest part of wild population lives at the Białowieża National Park area. In general, hematology data in European bison is scanty and cow's results are usually used as a reference. Studies in American bison (*Bison bison*) showed, however, the range for total white cell numbers was shifted lower for bison as compared to cattle. Lymphocyte and monocyte numbers were shifted in the same direction as well (1). Gasometry results in bison's blood were not found in available literature. The aim of present study was to start building a blood results database for European bison.

Material and methods

Blood samples were collected during routine immobilization for health monitoring in 7 European bisons in two locations, Kruklanki Forest District (n = 3, 1 - male, 2 - females) and Białowieża National Forest (n = 4 - females). Animals (age range: 3-16 years) were immobilized using etorphine and acepromazine combination administered intramuscularly using pneumatic rifle. Blood samples were withdrawn in August 2023 (n=1) and late November 2023 (n=6). Blood analyses were performed using veterinary automatic hematology analyzer (MINDRAY BC-5000vet) and portable gasometer (EDAN i15). Data are shown as their means and SD.

Results

Clinical examination did not show any signs of inflammation nor disease. Hematology results of one animal sampled in August agreed with earlier study in Białowieża National Park (Gill, 1999) as well as were fixed within a physiological range of adult cows (Meyer and Harvey, 2004). In all blood samples taken in late November, however, the white blood cell (range, 21-85 x109/L), neutrophile (range 4,6-24 x109/L), and lymphocyte (range, 19-61 x109/L) numbers were higher as compared to results obtained in August, and the ranges reported by Gill (1999) and Meyer and Harvey (2004). Comparing to cow profiles, all bison samples showed slightly elevated erythrocyte indicators (MCV, MCH and MCHC), as well as blood glucose (5,95±1,0 mmol/L) and lactate concentrations (1,40 ±0,47 mmol/L). Blood gasometry results were not much different from those reported in dairy cows (Kupczyński and Chudoba-Drozdowska, 2002).

Conclusions

It is too early to draw definitive conclusions regarding blood results bearing in mind a limited number of animals studied. However, we suggest that constructing a new data base for European bison like it was done for American bison already (ref. #1.) is more appropriate than using cattle data. Whether the late autumn season had indeed an impact on white blood cell profile it also needs more investigation.

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1047 - Can the ATP bioluminescence meter be used as an on-farm tool for assessing the microbiological quality of drinking water?

Author: Geert Hoflack, P.P.A. Penterman, N. Botteldoorn, Geert Vertenten, Bart Sustronck, Pleun Penterman

Objectives
Water is one of the most important nutrients for cattle to sustain live and optimize growth and productivity. Therefore, an adequate supply of high quality drinking water is essential for optimal performance and animal welfare. One aspect of water quality is the degree of microbiological contamination which is usually evaluated using laboratory procedures. The adenosine triphosphate (ATP) bioluminescence meter has been advocated as a simple and useful tool for the evaluation of the degree of microbiological contamination of surfaces and liquids. The objective of the present study was to examine whether an ATP bioluminescence meter can be used as an on-farm tool to assess the degree of microbiological contamination of drinking water.
Material and methods
A total of 153 drinking water samples from different cattle farms in Belgium that were submitted to the reference laboratory (DGZ, Torhout, Belgium) were included in the study. All samples were stored at 4°C until processing. The total bacterial cell count (TBCC) was determined at 22°C using an ISO 6222 standard method. The ATP bioluminescence, expressed as the number of relative light units (RLU) for each water sample, was obtained using AquaSnap™ Total swabs and a SystemSURE Plus meter (Hygiena™, California, USA). On each occasion, the mean of three consecutive ATP bioluminescence measurements was calculated and used for analysis. Optimal cut-off points for the ATP bioluminescence method to classify drinking water as 'microbiologically suitable' (TBCC < 100.000 cfu/ml) or 'microbiologically unsuitable' (TBCC > 100.000 cfu/ml) as drinking water were determined. TBCC values were Log10 transformed before analysis. Statistical analysis was performed in R (R Core Team 2022).
Results
The median TBCC of the drinking water samples was 1,34 Log10 cfu/ml and ranged from 0 to 5.48 log10 cfu/ml. The median ATP bioluminescence of the drinking water samples was 7 RLU (range 0 – 1113 RLU).
The optimal cut-point for the ATP bioluminescence method was 8 RLU (sensitivity 100%, no false negative samples) to classify drinking water samples as 'microbiologically suitable'. The optimal cut-point for the ATP bioluminescence method was 650 RLU (specificity 99%, 1% false positive samples) to classify drinking water samples as 'microbiologically unsuitable'.
Conclusions
The results of this study indicate that ATP bioluminescence measurements can be used as an on-farm screening tool to evaluate the microbiological quality of drinking water. Water samples with an RLU of less than 8 can be considered as 'microbiologically suitable' whereas, water sample with an RLU of more than 650 should be considered as 'microbiologically unsuitable' as drinking water. Water samples with an RLU between 8 and 650 should be submitted to an appropriate laboratory for microbiological quality assessment.

1614 - Do goats feel?: an approach to sentience and emotions

Author: Diana Patricia Martínez Albarrán, Ulises Jesús Bautista Pérez

Objectives

The word "feel" refers to experience, to perceive something through the senses in a certain state or situation that can be external or internal. While we can derive that goats have the capacity to feel, it's feasible to understand what they feel since the "sentience" means.

The present resume aims to attend the sentience of goats through the recognition of emotions, cognitive and affective states, to can be recognized and used as an instrument in the determination of the animal welfare.

Material and methods

Sentience is the capability to have positive or negative feelings, which includes having some degree of awareness of themself and their environment, as well as the cognitive ability to process and evaluate the actions of others in relation to themself and others, remember actions and consequences, generate and express emotions, evaluate risks, and plan to achieve objectives or goals.

Goats possess the capacity to feel pain in a sensory or emotional way being an unpleasant experience which can lead the individual to suffering. This response to pain is aversive and is a way of alerting and moving the individual away from the painful source.

Although it's difficult to demonstrate pain sensitivity in goats, it can be perceived since they have nociceptors and nerve that conduct stimuli to the brain where are processed, and it is also manifested by changes in their behavior and posture. The credibility of pain manifestations observed from human to human is based on non-verbal behaviors rather than verbal ones. In goats it is possible to measure some changes through zoosemiotics science, which is also a broad topic, but for now we will only focus on emotions and some cognitive and affective states.

Results

Although there is no universal definition, an emotion is an immediate and intense physiological reaction of short duration to an event, which allows the individual to execute behavioral reactions. Unpleasant emotions have a function of social adaptation and personal adjustment. In general, they represent the basic tools for survival, learning and adaptation.

Basic emotions in goats:

Sadness. Appears in situations of loss or separation, helplessness, isolation and pain and grief. It is an unpleasant sensation associated with the negative, but it favors social rapprochement and decreased activity.

Joy. Emotion of achievement, feeling of satisfaction. It favors cognitive performance, problem solving, as well, as learning, memory and exploration. Generates positive attitudes, strengthens bonds between individuals, including interspecies.

Fear. When danger is perceived, although a sudden visual, olfactory or auditory stimulus may elicit a startle response, fear depends on a more complex analysis in which current sensory information is compared with memories of previously experienced events. Fear elicits an intense aversive response that causes goats to flee, fight or freeze.

Anger. A feeling of intense displeasure at situations in which the goat is unable to control events, due to actions of other individuals or changes in the environment. It involves aggressive and violent behavior and may be preceded by other feelings such as frustration, pain, fear and anxiety. It prepares for self-defense and involves emotional memory.

Disgust. Occurs in the face of unpleasant stimuli, detonates rejection and escape behaviors to protect themselves.

Surprise. Neutral emotional reaction, it's the fastest, it's produced before a novel, strange and unexpected situation. It also fades quickly, eliminating the residual activity of the central nervous system, way to emotions congruent with such stimulation.

A feeling or affective state is a cerebral construction from the sum of emotions and preconceived giving concepts based on experiences that when repeated make animals modify their behavior, these are of long duration. In goats we can observe some of them such as suffering, frustration, empathy, hope.

One of the most evident signs in goats to recognize these affective states and emotions are vocalizations, however, changes in their behavior and posture can be indicative of situations they are going through.

Conclusions

It's important to know and recognize basic emotions as important elements for the determination and evaluation not only of the communication and interaction that exists between goats and their environment, it has even been pointed out that welfare isn't simply the absence of negative emotions, but also the presence of positive emotions, and considering them can change the way we communicate with goats.

1777 - Seropositivity of bovine brucellosis and its effects on reproduction in cross breed dairy cattle in addis ababa and the surrounding dairy shades

Author: ANTENEH MERSHA YENEHUN

Objectives
The objectives of this cross-sectional study, conducted in Addis Ababa and the surrounding dairy sheds (Selale and Sebeta), were to determine the seroprevalence of bovine brucellosis, assess the influence of various risk factors on its prevalence, and estimate the impact of bovine brucellosis on selected reproductive parameters.
Material and methods
A one-stage cluster sampling method was employed to collect 902 samples from 83 herds, testing them serologically for brucellosis using the Rose Bengal Plate Test. Positive samples underwent further testing via the Complement Fixation Test. Additionally, a questionnaire survey was conducted to gather relevant information. The gathered data were inputted and organized using Microsoft Excel before being transferred to STATA Version 12 (Stata Corp., College Station, TX) for comprehensive analysis, aiming to evaluate the impact of different factors on seroprevalence.
Results
The overall individual and herd-level seroprevalences were 0.9% (8 out of 902) and 7.2% (6 out of 83), respectively. All seropositive animals were from Addis Ababa dairy farms. Age and breed significantly influenced the comprehensive seroprevalence of bovine brucellosis across the study areas ($p<0.05$). However, the stage of lactation, pregnancy status, and the source of establishment or replacement stock showed no significant effect on the overall seroprevalence of bovine brucellosis ($p>0.05$). The seroprevalence was higher in Jersey crosses (8.33%) than Holstein crosses (0.79%) and in animals above 2 years of age (1.71%). All seropositive animals were in herds with more than 10 animals, predominantly females, with one or more parity. Additionally, the majority of seropositive animals (7 out of 8) were non-pregnant. Reproductive disorders, including abortion, retention of fetal membrane, repeat breeding, and other issues such as a prolapsed uterus and weak calf, were significantly associated with the seroprevalence of brucellosis ($p<0.05$). Moreover, breeding methodologies used and the presence of a separate calving pen were significantly associated ($p<0.05$) with brucellosis positivity. Conversely, examination before purchase and awareness about brucellosis were significantly associated with seropositivity ($p>0.05$). The disease was found exclusively in herds that encountered aborted material and/or fetal membrane and practiced unsafe assisted delivery. There was a significant association between the number of services per conception and seropositive cows ($p<0.05$), although a significant association was not observed between calf crops per production year and seropositivity.
Conclusions
In conclusion, a notably low seroprevalence of bovine brucellosis was observed in Addis Ababa and the surrounding dairy sheds. Recommendations include the slaughter of positive reactors and enhancements in management and husbandry practices to mitigate the impact of brucellosis. The importance of continued monitoring and preventive measures is emphasized to ensure the sustained health and productivity of dairy cattle in the region.

Education in ruminant veterinary medicine and production

1345 - Transhumance: bringing the student closer to the rural world

Author: MARIA ANGELES RAMO GIL

Objectives

In the wake of UNESCO's profound recognition of transhumance as an Intangible Cultural Heritage of Humanity, the recent meeting in Kasene (Botswana) marked a significant milestone in preserving and celebrating this age-old practice (source: Europa Press, last accessed on December 15th, 2023).

The UNESCO declaration in December 2023 acknowledges transhumance as an integral part of the Intangible Cultural Heritage of Humanity, underscoring its enduring importance. For centuries, the "cordeles," "cañadas," and "veredas" (ravines and paths in Spanish) have served as conduits for herds, fostering a sustainable and viable economy. These historical routes, as highlighted in the White Paper on Transhumance (2013), not only connect with the territory but also contribute significantly to the preservation of cultural heritage.

Revitalizing transhumance in the 21st century requires a delicate balance between innovation and tradition. An accurate analysis of its current state, through specific tests, is imperative to identify aspects that can receive effective and mutually complementary impulses. Recognizing transhumance in research, education, and mass media is as crucial as addressing its biological, veterinary, technical, legal, and infrastructural facets (White Paper on Transhumance, 2013).

Material and methods

The educational activity performed since 2012 by the Schoolf of Veterinary Sciences of the Univesity of Zaragoza (Spain) consists of a 24-day journey accompanying a flock of 3,000 Merino sheep from Guadalaviar (province of Teruel)(to Vilches,province of Jaéin) covering 500 kilometers and traversing multiple Spanish autonomous communities. The experience involved a total 34 students, divided into four groups spending six-day periods, providing them with diverse perspectives on the veterinary profession and livestock sector.

Daily gatherings and discussions addressed various issues, fostering knowledge generation about different aspects of livestock activity. This year's focus on toxic plants impacting livestock during displacement showcased the practical relevance of the transhumance experience. Real-time monitoring of route-related data through GPS carried by the animals themselves added a technological dimension to the traditional practice.

The herding tasks, guided by the shepherds, were documented on an open blog (www.conlatrashumancia.blogspot.com) and Instagram (Trashumancia.unizar), providing real-time insights into this immersive experience.

Results

At the end of the activity, the students are able to

- Organization of transhumance shifts in terms of dates, transportation, locations, etc.
- Purchase of all the necessary material (tents, mats to protect the tents in winter, tarps, kitchen tent, etc.).
- Hiring and maintaining vehicles for 4 weeks, including the following round trips
- Selection of the students who will attend weekly.
- Search for responsible personnel among the teaching staff.
- Manage the activity with the shepherds.
- Coordination with the media to bring said activity to the newspaper, radio, television, etc.
- Collaboration with companies to carry out studies and promote knowledge of transhumance to the public, as well as digitalization of livestock trails.

- Financial management of the activity, which includes: payment to shepherds, material, transportation, daily allowances, etc.

Conclusions

Participation in transhumance offers veterinary students a unique opportunity to improve their academic training by immersing themselves in the rural environment. Beyond theoretical knowledge, the experience enriches their practical understanding of various ruminant species, allowing them to witness and address livestock challenges firsthand.

Transhumance engages students in rural life, exposing them to the daily activities of farmers and providing occasions for hands-on veterinary work. This immersive experience often plays a pivotal role in shaping students' career aspirations within the veterinary field.

Moreover, transhumance fosters interdisciplinary relationships, allowing students to learn from and collaborate with shepherds, teachers, and other professionals in veterinary medicine. The broader perspective gained through this activity enhances students' understanding of the rural sector's significance in terms of conservation, animal welfare, and sustainability.

In summary, transhumance stands as a valuable addition to veterinary education, offering students a more comprehensive and enriching view of the rural world and livestock farming. The UNESCO recognition further underscores the importance of preserving and perpetuating this cultural heritage for future generations.

1463 - Who “fits” in farm animal practice: A mixed methods study of veterinary student perceptions

Author: Emily Payne, John Remnant

Objectives
Bovine and production animal veterinary workforce shortages are reported globally. This is a multifactorial problem, requiring multiple solutions. One of the approaches to help mitigate the shortage is encouraging more veterinary graduated to consider a career working with production animals. Previous work has shown that a feeling of “fitting in” was a factor determining veterinary students’ interest in a farm animal careers [1]. The aim of this study was to identify factors that affected the perception of “fit” in veterinary students.
Material and methods
Data from a survey of 1,146 veterinary students in the UK and Ireland were analysed using a mixed methods approach. Respondents were asked for some demographic data, and their level of agreement with the statement “I feel able to pursue a career in farm animal practice”. These quantitative data were analysed using multiple regression to identify associations between perceived fit and demographic characteristics. A concurrent qualitative analysis was conducted on the free text responses to the same question, using thematic analysis.
Results
Those with a female gender, marginalised ethnic identities, and those from an urban or suburban background had significantly less agreement with the statement “I feel able to pursue a career in farm animal practice” than male, white, rural background veterinary students.
Thematic analysis identified six themes: Career opportunities, nature of farm animal veterinary work, individual experiences, relationships and interactions, expectations and perceptions, and no perceived barriers.
Conclusions
This study identifies that some demographic groups feel less able to pursue a career in production animal practice [2]. This can be related to their experiences in this sector, the interactions they encounter, the nature of the work, the career opportunities they perceive will be available, and their expectations. There were also respondents that perceived no issues here, despite the quantitative data demonstrating barriers.
Addressing the issues described in this study could be an important step towards mitigating the production animal veterinary workforce shortage.

References
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1436 - Global perspectives on education of veterinary students on antimicrobial stewardship

Author: Sarah Wood

Objectives
As new veterinarians enter production animal practice it is imperative that they have the confidence to prescribe antibiotics appropriately and a working knowledge of antimicrobial stewardship (AMS). The importance of AMS is widely recognised as critical in minimising the risk of antimicrobial resistance (AMR) within both veterinary and human medicine. The production animal veterinary sector has been a particular area of focus for improved AMS due to concerns about resistance developing from antimicrobial use in food producing animals. Inclusion of the topic of AMR and AMS is a necessary feature of veterinary education worldwide, to ensure preparedness for responsible prescribing at graduation. In recent years studies have been published in the peer reviewed literature from across the world exploring veterinary students' knowledge, attitudes and perceptions of AMS. The aim of this study was to review the published literature to identify similarities and differences in veterinary students across the world and to identify what changes to the veterinary curricula are needed to ensure future veterinarians are prepared to tackle AMR.
Material and methods
A structured literature search of the peer reviewed literature was performed to identify relevant papers. To ensure the focus was on veterinary education search terms were as follows "antimicrobial" OR "antibiotic*" AND "veterinary" AND "student*". References and citations of relevant papers were also reviewed to ensure a thorough search of the literature.
Results
19 papers included data sets from veterinary students exploring knowledge, attitudes and perceptions of AMS, all published between 2018 and 2023. These papers covered institutions providing a veterinary programme across the world; with papers in Europe (9), Africa (5), North America (2), Asia (2) and Australasia (1). All papers concluded that there are improvements to be made in veterinary education towards preparedness for responsible antimicrobial prescribing and stewardship. Attitudes towards a veterinarian's role in AMS varied across countries. When asked whether vets contributed to prevention of AMR as few as 50% of participating students agreed with such a statement in some countries, but up to 98% agreed in other national studies. Similarly, confidence in knowledge or assessed knowledge of antimicrobials, including classification of products, awareness of characteristics of use and application to clinical scenarios varied across the studies. Across the published work students indicated they would benefit from additional teaching on aspects of antimicrobial use, AMR and AMS. Some papers offered specifics to improve teaching of this topic; suggestions included use of problem-based learning to link pre-clinical and clinical teaching, practical application. Suggested resources included use of infographics and visual materials, and improved institutional level guidance on prescribing.
Conclusions
There is still work to be done to ensure veterinarians of the future take an active role in AMS. Veterinary students globally do not consistently recognise their role in prevention of AMR and protection of global health. Veterinary education is central to ensure veterinarians can play an active and effective role in AMS, and curricula can be reviewed in light of these findings to ensure there is practical application of theoretical teaching to ensure graduates are prepared for responsible decision making when prescribing antimicrobials.

POSTER PRESENTATIONS

Infectious diseases

1026 - Prevalence and risk management of Bovine Corona-Virus infections in Austrian dairy farms

Author: Harald Pothmann, Marlies Dolezal, Nina Hye, Marlies Schönecker, Anna Catharina Berge, Geert Vertenten, Marc Drillich

Objectives
The objective of this cross-sectional field study was to estimate the farm prevalence of Bovine Corona-Virus (BCV) in small and medium sized Austrian dairy farms, and to predict farm-level risk factors in management that are associated with BCV infections in calves and cows.
Material and methods
Ten conventional dairy farms in Austria with 45 to 350 cows were enrolled in this study. On each farm, a questionnaire consisting of 181 questions was used to determine various management factors, e.g. colostrum management or husbandry, and biosecurity measures, e.g. vaccination against BCV or trading policy, resulting in a biosecurity score (0-100) of each farm (www.biocheck.ugent.be). Bulk tank milk of each farm and serum samples from pre-weaned calves, recently weaned calves and fresh cows were analyzed for the presence of BCV antibodies by using ELISA (Monoscreen, Bio-X Diagnostics, Rochefort, Belgium). Nasal and fecal swabs were collected for RT-PCR analyses (Kylt® Bovine Coronavirus, SAN Group Biotech, Germany). Prevalence of BCV was calculated within farms and within age category of animals. Generalized mixed logistic regression models with binary outcomes (results of ELISA, PCR) were performed to determine odds ratios for BCV infections for the fixed effects of age category and a covariate of biosecurity score. Farms were modelled as random intercept. Significance was set at an alpha level of 5% (after Tukey multiple testing correction for age category).
Results
Bulk milk samples were positive in all farms. Individual serology in fresh cows, weaned and pre-weaned calves, resulted in 98.0, 76.3, and 97.4% antibody positive animals. Presence of BCV was confirmed in 3.8, 7.5, and 18.4% of nasal and in 3.8, 11.3, and 15.8% of fecal samples (adults, weaned, pre-weaned calves). There was no significant difference in recovery of viral nucleic acid from nasal versus fecal swabs. Pre-weaned calves, however, had significant higher odds for being PCR positive compared with adult cows (OR 50.4 nasal and 48.9 in feces, resp.), while the odds for weaned calves versus cows were 26.7 times higher (nasal swabs).The average biosecurity score on farms was 58 and ranged from 52 to 63. The probability of a positive PCR sample (nasal, fecal) at an average biosecurity score was 15.8 and 13.4% in pre-weaned calves and relatively low in cows (0.4 and 0.3%, resp.).
Conclusions
All herds were seropositive to the BCV. Within herds, most animals were seropositive independent from age. Pre-weaned calves showed greater respiratory and enteric BCV

abundance compared with weaned calves and adults. The biosecurity check revealed that Austrian farms should aim at improvements to limit potential BCV infections. Pre-weaned calves were at highest risk and would therefore benefit most from implemented measures.

1049 - Seroprevalence of BRD pathogens on Dutch problem farms: is bovine coronavirus involved?

Author: Henk Kuijk, P.P.A. Penterman, H. Swam, Geert Vertenten, Bart Sustronck

Objectives

Bovine respiratory disease (BRD) remains a leading cause of morbidity, mortality and economic loss to the cattle industry. In the last decade it has become obvious that besides bovine respiratory syncytial virus (BRSV) and bovine para-influenza type 3 virus (PI3), bovine corona virus (BCoV) can be involved as viral agent in this BRD complex. The goal of the present study was to obtain data on the seroprevalence of BCoV on BRD problem farms in The Netherlands and to compare this seroprevalence to those of well-established respiratory pathogens.

Material and methods

A cross sectional study on 63 Dutch dairy herds was conducted from October 2022 until March 2023. The selected herds did not implement any vaccination program against BRD in their young stock and all recently experienced BRD problems in young calves (less than five months old). On each farm serum samples of five randomly selected, three to six months old calves were collected. This age category was selected to avoid the possible interference of maternal antibodies. The samples were analysed in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands) for antibodies against BRSV, PI3, BCoV, Mannheimia haemolytica (Mh), and Mycoplasma bovis (Mb) by ELISA. An in-house test was used to measure Mh and BRSV antibodies, whereas a commercial kit from IDEXX was used for PI3 antibodies. BCoV and Mb antibodies were determined using commercial Bio-X ELISA test kits. For each BRD pathogen herd-level and calf-level seroprevalence was determined and compared.

Results

In 56 of the 63 BRD problem farms (88.8%) at least one animal with antibodies against BCV was detected. BRSV antibodies were detected in 27 of the 63 problem herds (42.8%). The herd level seroprevalence for PI3, Mh and Mb was, respectively 80%, 71.4% and 23.8%.

273 of the 315 calves tested (86.7%) had antibodies against BCV. Nearly half (48.7%) of the calves seropositive for BCV had a high to very high titer in antibodies, indicating a possible recent BCV infection. The calf level seroprevalence for BRSV was 22.4% compared to 63.6%, 64.3% and 18.5% for PI3, Mh and Mb, respectively.

Conclusions

This study clearly shows that BCoV was highly prevalent on BRD problem farms in The Netherlands. In 89% of the BRD problem herds BCoV seropositive calves were detected and nearly 50% of those calves showed evidence of a recent contact with the virus. On the other hand, on Dutch dairy farms with BRD problems mycoplasma seems to be of minor importance since in only 15 of the 63 herds, calves with Mb antibodies could be detected.

1051 - Intranasal vaccination of calves at day of birth with a live attenuated vaccine against BRSV and PI3 and a live attenuated vaccine against respiratory coronavirus.

Autor: Geert Vertenten ., Mark H. van Rooij, Mélodie Schmitz, Joris M.H. Meessen, Pieter A.W.M. Wouters, Mieke P. Vrijenhoek, Birgit Makoschey, Klaas Okkinga

Objetives

Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza 3 Virus (BPIV3) and Bovine Corona Virus (BCoV) are considered the major viral pathogens the BRD complex. In the studies reported here, the safety and immunity of 2 commercial intranasal live attenuated vaccines (a BRSV-BPIV3 combination vaccine and a BCoV vaccine) were investigated in calves at day of birth.

Material and methods

The investigations comprised one safety study (#1) and three efficacy studies (#2–#4). All calves were deprived from the intake of colostrum and vaccinated intranasally at the day of birth with the live attenuated BRSV/BPIV3 combination vaccine Bovilis® INtranasal RSP® Live (MSD Animal Health) (studies #1–#3) and Bovilis® Nasalgen®-C (MSD Animal Health) (study#1 and #4).

In the safety study (#1), ten calves were vaccinated with both vaccines at day of birth and revaccinated two weeks later. In the efficacy studies, the animals were vaccinated with the BRSV/BPIV3 combination vaccine (six animals in study#2 and five animals in study#3) or the BCV vaccine (study#4, five animals). Three animals (two in study#1 and one in study#2) were excluded from evaluation of results because they had antibodies against at least one of the vaccine viruses.

At six (study#2), seven (study#3) and five (study#4), days post vaccination, the calves were experimentally infected with BRSV (study#2), BPIV3 (study#3) and BCV (study#4) field isolates by aerosol. The animals in the safety study (study#1) were not challenged. Throughout the study, animals were monitored for any adverse effects of the vaccination and clinical disease following the challenge infection. Samples were collected and tested for RNA specific for the respective vaccine and challenge viruses. The animals in study#2 were euthanized fourteen days after the BRSV challenge infection and a necropsy was performed to observe the presence of possible lung lesions.

Results

Safety Study

The frequency and extent of symptoms of abnormal health was low, indicating that the vaccines were very well tolerated.

Challenge Studies

Mild to moderate signs of upper respiratory tract disease such as nasal discharge and coughing were the predominant clinical signs observed after challenge infection. In all three studies, the average scores in the vaccinated groups were lower than in the control groups. The differences were notable, however not statistically significant due to considerable variation within the groups. With only a few exceptions, the temperatures remained below the upper physiological limit (39.5°C).

After BRSV challenge infection, macroscopical lung lesions were noted in five (out of six) control animals but only in two (out of five) vaccinated animals. The difference in

average lung consolidation scores was notably in favour of the vaccinated animals but did just not reach significance.
All animals shed challenge virus with nasal discharge for at least one day. For all three viruses, the virus load in nasal swabs was significantly lower in the vaccinated group.

Conclusions

Simultaneous intranasal vaccination at day of birth with a live attenuated commercial vaccine against BRSV and PI3 and a live attenuated commercial vaccine against BCoV was safe and effective

1052 - Serological prevalence of five common Bovine Respiratory Disease (BRD) pathogens in Great Britain

Author: Geert Vertenten ., Liz Cresswell, Alexandra Ashworth, Katherine Baxter-Smith, Rebecca Cole, Eleanor Rawson

Objectives

Bovine Respiratory Disease (BRD) is one of the leading causes of calf morbidity and mortality, estimated to cost the UK farming industry £80 million per year. It is recognised to be a multifactorial disease requiring a multifaced approach to control. After infection with respiratory pathogens a positive serum antibody response can be detected after 4-6 weeks. The objective of the study was to identify the prevalence of seroconversion to different BRD pathogens in order to understand their proportional contribution to BRD in British cattle herds between November 2020 and November 2022.

Material and methods

A total of 255 calves were sampled on 51 different farms selected by convenience sampling across Great Britain. Five blood samples were taken per farm from unvaccinated calves between 3-6 months of age who were not in the acute stages of respiratory infection. Enzyme Linked Immunosorbent Assay (ELISA) testing (IDEXX) was undertaken for antibodies against Bovine Coronavirus (BCoV), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* (MANH), *Mycoplasma bovis* (MB) and Parainfluenza Virus 3 (PI3).

Results

Positive antibody responses were most commonly detected against BCoV, PI3 and MANH. Eighty four percent of farms returned positive antibody responses to three or more pathogens. There was little variation in the distribution of serological positivity by geographical area. There was some seasonal variation in seropositivity against different pathogens, although low numbers of farms make analysis difficult and further data collection is ongoing. Bovine Coronavirus seropositivity remained high throughout the year, with over 95% of farms tested in spring, summer and winter returning positive results. Bovine Coronavirus was most commonly detected with two other pathogens, with antibodies against MANH and PI3 respectively being detected on 91% of farms which also tested positive for BCoV.

Conclusions

All five pathogens were highly prevalent in calf populations across Great Britain. Seroprevalence was highest against Bovine Coronavirus, *Mannheimia haemolytica* and Parainfluenza 3. It is most likely that farms have more than one pathogen contributing to BRD in their calf populations. These findings indicate that a multifactorial approach is needed for control of BRD, comprising factors which reduce the environmental pathogen load (such as ensuring good hygiene and biosecurity) and improve calves' immunity (such as through ensuring good levels of colostral passive transfer and vaccination).

1056 - The impact of a modified live Mycoplasma bovis vaccine on antibiotics usage on a French feedlot

Author: Stéfanie Bernheim -, Alfredo Zanini, Leentje Dreesen, Ely Benere, Vanessa Dablin, Barbara Descamps, Suman Mahan, Abhijit Gurjar, David Asper, Véronique Moulin

Objectives
Bovine respiratory disease caused by *Mycoplasma bovis (Mb)* has posed a perpetual torment for cattle producers. *Mb* infections in calves cause major economic losses and extensive use of antibiotics (AB). [Protivity®, Zoetis] is a Modified Live *Mb* vaccine available in France since 2022 for vaccination of healthy cattle of 1 week of age and effective in reduction of clinical signs and lung lesions. If AB are required within 15 days before or after vaccination , preference should be given to those with no anti-*Mycoplasma* activity. The impact [of Protivity®] on Antimicrobials Use (AMU) was evaluated in a French *Mb* infected feedlot.
Material and methods
1725 limousin calves fattened for 150 days were monitored in 2022-2023, after an *Mb* outbreak in 2021. On a weekly basis, 8-12 month old calves were comingled and sorted to groups of 11, and quarantined for 3 weeks. From March 2022, 2 [Protivity®] doses , 21 days apart, were added to the vaccination protocol for the new arrivals [Rispoval® 3 (RSV, Pi3V, BVD, Zoetis), Hiprabovis somni LKT® (*Mannheimia haemolytica, Histophilus somni*, Hipra)]. Five months were needed to immunize all 450 calves on site. Treatments were recorded with date, product name, body weight and dosage. Three semesters were compared: 22S1 (01-06 2022 with ongoing *Mb* outbreak, *Mb* vaccination from 0 to 67%), 22S2 (07-12 2022, 67% to 100%) and 23S1 (01-06 2023, 100%). Two indexes estimated the antimicrobials level of exposure of 525 to 575 animals/semester: the AMU/PCU (Population Correction Unit) in mg of active substance per kg of body weight with an average of 580 kg at slaughter and the ALEA (Animal Level of Exposure to Antimicrobials) at farm level [(total treated body weight)/(number of animals)*(body weight at slaughter)].
Results
ALEA for 22S1 (outbreak and partial vaccination) was 0.9. It decreased to 0.3 for 22S2 and 0.1 for 23S1 (level never reached before). The AMU/PCU was 13.1 mg/kg for 22S1, decreased to 3.9 for 22S2 and 3.7 for 23S1, The differences between semesters for both indexes were significant ($p<0.0001$).
Conclusions
ALEA and AMU/PCU are quantitative approaches of a global exposure to AB. Both indexes were reduced after *Mb* vaccination which demonstrates that Protivity® contributed to this significant decrease in AMU. An even more accurate assessment of this reduction could be the use of Daily Defined Doses for animals (DDDvet) vet which will be the next step of this evaluation, after 3 years of vaccination.

1063 - Pathogens associated with neonatal diarrhea in dairy calves in México

Author: J.C. Limon Varela, D. Val Arreola, J. M. Swinkels, J. A. Garcia Escalera

Objectives

Neonatal diarrhea (ND) remains the most important cause of mortality in calves under one month of age, with substantial economic losses in dairy farms worldwide. Besides the short-term cost of treatment and the associated extra labor, ND has an impact in the long term, such as an increase in the age at first calving and reduced milk yield. The objective was to determine the frequency of the different causative agents of ND in neonate Holstein calves in the North and Central regions of Mexico, to be able to establish preventive programs according to the needs of each region.

Material and methods

The study was carried out in 57 dairy farms. An average of 8 samples per farm were taken in the period February 2018 to July 2023 from calves of 1-21 days old with clinical signs of diarrhea before receiving antibiotic treatment. The samples were collected from the calf's rectum in order to avoid contamination, were homogenized and processed directly on farm. The test result was read after the required 10-minute waiting period.
The records of 408 diagnoses made in feces of calves with ND, carried out with a commercial rapid immunochromatography test (RAINBOW CALF SCOURS- BIO K 360, BioX diagnostics) aimed at identifying the antigen, were used to establish the proportion of positive cases and frequency of the pathogen's *Cryptosporidium parvum*, Rotavirus, Coronavirus, *E coli* and Clostridium *spp.*. A binary reversal statistical analysis was performed to determine the probability of infection for each of the pathogens according to the age of the calves.

Results

The most frequently identified pathogens were *Cryptosporidium parvum* (72%) and Rotavirus (29%), followed by Clostridium *spp.* (10%). The highest proportion of positive cases for these 3 pathogens was between 5 and 15 days of age. *E. Coli* had a relatively low frequency (4%) and the lowest frequency was Coronavirus (1%). The frequency of positive cases between the years that the study lasted was variable. *Cryptosporidium parvum* and Rotavirus observed the highest frequencies in 2018 and 2019, while in 2022 Clostridium and Coronavirus showed a higher frequency of positive cases. The results also show variation within years, the Fall-Winter showing the highest frequencies for the 5 pathogens, particularly for Clostridium *spp.* and *E. coli*.
By geographic region, Cryptosporidium and Rotavirus were observed more frequently in the central states of Mexico, while Clostridium *spp.* and *E. Coli* were more frequent in the northern states. The binary regression analysis showed that the highest probability (0.6) of infection was with *Cryptosporidium parvum*, starting from day 5 of age and increasing with the animal´s age up to 15 day of age, while in the rest of the pathogens evaluated, the probability of infection decreases with age. For Coronavirus and *E. coli* the probability of infection is significantly reduced from day 5 of age and for Rotavirus and Clostridium *spp.* from day 15 of age.

Conclusions

Pathogens causing diarrhea in neonatal dairy calves in the first 21 days of life, were mostly *Cryptosporidium parvum*, Rotavirus and Clostridium *spp.* between 5 and 15 days of age and their prevalence varied between regions of Mexico, years and season. *Cryptosporidium parvum* was the most frequently identified pathogen and the incidence increased with the animals age.

1064 - Retrospective study of incidence of cattle respiratory disease pathogens from clinical laboratory samples submitted by UK veterinary practices.

Author: Katharine Denholm, Katharine Baxter-Smith, Paul Burr

Objectives

The objective of this study was to explore the prevalence and seasonality of respiratory pathogens in the UK (based on clinical case submission for laboratory PCR).

Material and methods

The study used retrospective data generated by a central Scotland laboratory using 407 clinical samples collected by 95 veterinary practices located throughout the UK between November 2020 and September 2022. Samples underwent RT-PCR (Applied Biosystems™ VetMAX™ Ruminant Respiratory Screening Kit & Applied Biosystems™ VetMAX™ Reagents) testing for bacteria (Mannheimia haemolytica, Pasteurella multocida, Histophilus somni and Mycoplasma bovis) and viruses (BHV-1 (IBR), PI3, BCoV and RSV). Pooled nasal swabs comprised 373 (91.6%) of the samples and 24 (5.9%) of the samples were lung tissue from postmortem examination, with the remainder of samples being unlabelled. Month of sample submission and age of animal was extracted from the submission forms. Whether the samples were submitted from beef or dairy enterprises was unrecorded on the submission forms. Results for each pathogen were reported semi-quantitatively as '-', '+', '++', '+++' by the laboratory based on the Ct threshold of the sample in each reaction. Statistical analyses were performed using descriptive spatial analysis (choropleth maps), chi-squared analysis, poisson and logistic regression modelling.

Results

The majority (77.6%) of samples had more than one bacterium detected and 17.7% had multiple viruses detected. Bovine Coronavirus (BCoV) was present in 38.6% of samples, IBR in 5.2%, *H. somni* in 41.3%, *M. haemolytica* in 61.7%, *M. bovis* in 39.1%, PI-3 in 11.5%, *P. multocida* 93.4% and BRSV in 21.6%. In comparison with the colder months of autumn and winter (September to February), the warmer months (March to August) were significantly associated with a lower odds of isolation for certain pathogens. Poisson models showed small but significant univariable associations between total viruses (coefficient= -0.01, SE=0.004; 95%CI=-0.02 - -0.003) and total pathogens (coefficient= -0.005; SE=0.002; 95%CI=-0.008- -0.002) and increasing weekly age.

This is an inherently biased population of results as it only comprises clinical samples submitted to a single UK laboratory and the data was analysed retrospectively

Conclusions

The majority of clinical bovine respiratory disease samples in the UK are multi-pathogenic. Bovine coronavirus has generally not been considered a significant contributing pathogen in the BRD complex in the UK, despite a growing body of evidence worldwide demonstrating both its presence and clinical importance in the syndrome,[1,2] however our data demonstrates it is frequently present. Warmer months and increasing age (in weeks) were significantly associated with a lower number of total pathogens isolated from clinical bovine respiratory disease samples.

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1066 - Long-term analysis of maternal bovine coronavirus antibodies: a prospective cohort study in Dutch Holstein-Friesian female breeding calves

Author: Henk Kuijk, M. Bakker, Pleun Penterman, Hanny Swam, Geert Vertenten, Bart Sustronck

Objectives

Bovine respiratory disease (BRD) is a multifactorial condition in which host and environmental factors predispose calves to infections with viral and bacterial pathogens. Serologic BRD screening to identify which major respiratory pathogens are circulating on a farm (BRD quick scan) is frequently being used on dairy farms in The Netherlands to guide practitioners and farmers in the implementation of improved BRD farm management routines and to adjust and fine-tune BRD vaccination programmes (Kuijk et al 2022).

In the last decades bovine coronavirus (BCoV) has gained momentum as possible viral pathogen involved in outbreaks of respiratory disease in calves. Necessitating the inclusion of BCoV in the routine BRD quick scan. In order to correctly interpret the result of a serologic BRD screening, including BCoV antibodies, a better knowledge of the evolution of maternal BCoV antibodies over time seems appropriate.

The aim of the present study is to obtain better insight in the evolution of maternal BCoV antibodies in the first 6 months of life of Dutch Holstein-Friesian female breeding calves. Additionally, the influence of dam vaccination against BCoV on those maternal antibodies in calves will be explored.

Material and methods

A prospective cohort study was conducted on a convenience sample of 5 Dutch Holstein-Friesian dairy herds, all belonging to the same veterinary practice. Inclusion criterion for the farms was a farm size of at least 100 milking cows. On each farm five new-born calves were randomly selected to be included in the trial. All calves received at least 3 litres of colostrum from their own dam in the first 24h after birth. The calves were blood sampled in the first month of life and each month thereafter for 6 consecutive months. The serum samples were analysed in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands) for antibodies against BCoV by ELISA using a commercial test kit (Bio K 392, Bio-X Diagnostics SA, Rochefort, Belgium). Data concerning dam vaccinations and occurrence of calf disease were obtained from the herd record keeping logbooks. The evolution of the BCoV antibodies over time was visually assessed. The association of dam BCoV vaccination and calf disease occurrence with calf BCoV antibodies was explored using generalized linear mixed models including farm as random effect. Statistical analysis was conducted in R (R Core Team 2022).

Results

This longitudinal study is ongoing. The preliminary data show that maternal antibodies against BCoV, as expected steadily decline during the first three months of life but can still be detected in Dutch Holstein-Friesian female breeding calves at that time. Maternal vaccination against BCoV has an important impact on the initial BCoV serum titer of the calves. Full results of this prospective cohort study will be presented at the congress.

Conclusions

This prospective cohort study revealed new insights into the evolution of maternal antibodies against BCoV in Dutch Holstein-Friesian female breeding calves. The findings

of this study will contribute to an objective and correct interpretation of the results of serologic BRD screenings (BRD quick scan) on Holstein-Friesian dairy farms.

References

Kuijk H., Swam H., Penterman P., and Vertenten G. (2022). Identification of BRD antibodies to install a tailor-made prevention plan on Dutch dairy farms. 31st World Buiatrics Congress Madrid, Abstract Book, volume 1, 230-231.

1075 - A Study to Determine equivalence of administering a live commercial BRSV/PI-3 intranasal vaccine to MDA+ve calves at Day 0 of life compared to 1 week of age

Author: Katherine Baxter-Smith, Graham Baird, Richard Cooper, Jude Roberts, Geert Vertenten, Egon Thesing

Objectives

Bovine Respiratory Disease (BRD) is a multifactorial disease of young and growing cattle. Affected animals are highly infectious and shed large quantities of virus and bacteria through nasal discharge. Clinical signs include reduced feed intake, fever, nasal discharge, coughing, strained breathing, lethargy and death.

Calves may be vaccinated early in life. However, calf immunisation may be adversely affected by interference from maternal antibodies (MDA). Consequently, the best time to vaccinate young calves against BRD is not evident. At present there is limited evidence demonstrating the efficacy of vaccinating MDA+ve calves intranasally before 5 days of age.[1]

In this study, we aimed to demonstrate the efficacy of using a commercial BRSV/PI3 vaccine [Bovilis INtranasal RSP Live] on the first day of life in colostrum-fed calves by comparing respiratory health in two groups of calves on commercial UK dairy farms, one group given the vaccine on D0 and another at 1 week of age (D7-10).

Material and methods

Seven dairy farms were recruited which fit the inclusion criteria. Calves were monitored daily by farm staff who recorded treatments, mortality, and morbidity. A veterinarian from the attending veterinary practice attended at pre-defined time points to weigh calves. Thoracic ultrasound was undertaken on 3/7 farms in calves of 6-8 weeks of age.

Calves (n.= 685) on 7 commercial UK dairy farms were equally and randomly split into one of two groups at birth (according to odd or even ear tag number), given 4-6L colostrum from their dams within 12 hours (therefore MDA +ve) then vaccinated with a live intranasal BRSV/PI-3 vaccine [Bovilis INtranasal RSP Live] on their first day of life (D0) or at one week of age (D7-10). Calves were not specifically challenged with virus; however, farms had a low level of respiratory disease and antibody testing of older youngstock confirmed recent exposure to field virus strains of BRSV and/or PI-3 on these farms.

Measurements included antibiotic treatments (taken from farm treatment records), weight gains (measured using weigh tapes), morbidity/mortality data and where practicable, thoracic ultrasound (TUS) scanning at 6-8 weeks of age. Calves were weighed in the first week of life, at 3-4 weeks of age and at 8 weeks of age. Colostrum was tested before administration to calves to ensure sufficient IgG levels. The dairy farms involved had at least 250 lactating cows, and a history of BRD that was deemed by the farmer to be 'under control' (10% prevalence or under). Dams were not vaccinated for BRD during pregnancy. Farms were free from *Mycoplasma bovis* and were either free from or had control measures in place (such as vaccination) for BVD and IBR, with a recent history of freedom from active infection. Infection status was determined by bulk tank antibody testing and blood testing a cohort of youngstock aged 3-6 months for antibodies to BVD, IBR (marker), and *M. Bovis.*

Weight data were analysed using a mixed-effect regression model, with farm modelled as a random effect. Parity, sex, month of birth were considered as covariates in the model. Binary regression models were used for analysis of secondary outcomes of proportion of animals treated for respiratory disease, mortality risk and lung score.
There was no negative control group due to health and welfare implications of not vaccinating a group of animals on a farm where other animals are vaccinated, thereby exposing the unvaccinated group to disease risk.
Administration of vaccine to animals at D0 or D7 was not blinded due to the unnecessary handling stress of administering a non-beneficial "treatment" (placebo) to young calves. However, the administrator was different to the study investigator to remove unconscious bias.

Results

Data are currently still under analysis.

Conclusions

Vaccinating MDA-positive calves on commercial UK dairy farms with a licensed BRSV/PI-3 vaccine [Bovilis Intranasal RSP Live] on the day of birth is equivalent in terms of morbidity, mortality and weight gain to vaccinating at 7 days of age. (conclusion might by adapted once all data analysed)

References:

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1076 - Efficacy of a live bovine herpesvirus type 1 marker vaccine under field conditions through total herd vaccination

Author: Clara Bourel, Geert Vertenten ., Andre Preto

Objectives

Many large cattle herds in France have been positive for Bovine Herpes Virus type 1 (BHV-1) for many years. In France, each year, 1 of 5 BHV-1 positive herds has an active circulation of the virus in the non-vaccinated animals as most infected farms only vaccinate the gE+ cattle[(1)]. Marker vaccines, deleted for the BHV-1 gE protein, enable serological differentiation between antibodies induced by vaccination and those resulting from infection with wild-type virus. Despite this advanced technology, most French herds infected with the virus vaccinate only their infected animals. Total herd vaccination is rare, and when it is introduced, it is often discontinued after 2 years.

The objective of this study was to evaluate the efficacy of a live marker vaccine against bovine herpesvirus type 1 (BHV-1) in the field on a BHV-1 positive farm.

Material and methods

Farm:

A dairy farm infected by BHV-1 virus since 2007 unsuccessfully trying to eradicate the virus for 13 years.

At the beginning of the study (September 2020), 270 animals older than 12 months were present on the farm. The seroprevalence for BHV-1 was 60 % (animals > 12 months old).

Antibody ELISAs

Every 6 months, the sera of the animals older than 12 months were analyzed by the Center for Diagnostic Solutions (MSD Animal Health, Boxmeer, The Netherlands). A commercially available ELISA that is specific for antibodies to gE of BHV-1 (HerdChek IBR gE Antibody ELISA; IDEXX) was used to detect animals infected with field virus. The test does not detect animals vaccinated with gE-deleted IBR marker vaccines, whereas cattle vaccinated with a non-marker (gE positive) IBR vaccine test positive.

Vaccine:

In addition of culling of the gE BHV-1 positive animals, the following vaccination schedule with a live IBR marker vaccine (Bovilis IBR Marker Live; MSD Animal Health) was implemented from September 2020 onwards, with monthly veterinarian visits:

- One intranasal vaccination of the calves older than 15 days
- One intramuscular vaccination of the calves older than 3 months, with a booster 6 months later
- Twice a year (spring and autumn) intramuscular booster vaccination of the heifers and cows.
- Within 15 days post-arrival: Intramuscular vaccination of the purchased cattle.

Results

During the first three years after implementation of the control program (culling and total herd vaccination), the incidence of new seroconversions decreased from 36,6% to 0%. As a result, the herd seroprevalence decreased from 59,7% in the fourth quarter of 2020 to 24% in the fourth quarter of 2022 and 0% in the fourth quarter of 2023 in the

lactating herd (≥ 12 months old). In January 2024, three years after the vaccination program was installed, the herd was certified free of BHV-1.

Conclusions

Total herd vaccination with a live IBR marker vaccine including culling of the infected animals, resulted in the eradication of BHV-1 under field conditions in a herd that has been infected for years.

1078 - Assessment of Pathogen Prevalence Frequency Associated with Bovine Respiratory Disease (BRD) in Mexican Dairy Heifers Using the Technique of Polymerase Chain Reaction (PCR)

Author: Marco Alberto Chapula, Pedro Rodriguez Fernandez, Alberto Garcia Escalera, Daniel Arreola Val, Ramon Echevarria Ponce

Objectives

In Mexico, Bovine Respiratory Disease (BRD) is of significant economic importance at dairy farms. One of the main challenges in addressing this disease is to achieve a precise diagnosis of pathogens causing BRD. Advanced laboratory tests, such as Polymerase Chain Reaction (PCR), have emerged as highly sensitive and specific diagnostic tool. This test enables a rapid and effective identification of the pathogens associated with BRD.The objective was to determine the prevalence of pathogens involved in BRD in dairy heifers using PCR test.

Material and methods

A total of 114 nasopharyngeal swabs were collected at multiple dairy farms located in the center and western parts of the country from Holstein calves of different ages exhibiting clinical signs of pneumonia prior to administration of any treatment. Swabs were shipped under refrigeration to the laboratory to perform identification of the pathogens using a PCR test. The PCR results were subjected to a Bayesian analysis to evaluate the probability of the frequency of each pathogen identified without assuming prior assumptions and considering a binominal distribution of 2x2.

Results

The pathogens identified were *Pasteurella multocida*, *Mannheimia haemolytica*, bovine coronavirus, *Mycoplasma bovis*, *Histophilus somni*, and bovine respiratory syncytial virus. The Bayesian analysis showed that *Pasteurella multocida* had the highest probability of 75% and CI (Credible interval)-95 6-82%, followed by *Mannheimia haemolytica* showing the probability of 25% and a CI-95 19-34%; coronavirus 18% with a CI-95 of 12-26%; bovine *Mycoplasma bovis* 19.8% with a CI-95 of 9-22%; *Histophilus somni* 15% with a CI-95 of 9-22%; and bovine respiratory syncytial virus 6% with a CI-95 of 3-12%.

Conclusions

Pasteurella multocida, *Mannheimia haemolytica*, *Mycoplasma bovis* and *bovine coronavirus* are the primary pathogens associated with BRD in this study. However, based on probabilities calculated by the Bayesian analysis, it seems that all pathogens tested in this study pose a relevant risk, which warrants creation of health preventive programs around these pathogens at Dairy farms in Mexico to reduce economic losses due to BRD.

1079 - Ecological Study on Bovine Brucellosis Vaccination for Control and Eradication in Colombia

Author: Bernardo Andres Guerrero Mateus, Alejandro Rico Mendoza

Objectives
Objective: To describe the impact of vaccination with the B19 strain in the bovine brucellosis control and eradication program conducted in Colombia between 2009 and 2021.
Material and methods
An ecological study of multiple groups was conducted to identify correlations between the presence of bovine brucellosis, the livestock slaughter survey, and the number of human diagnoses related to brucellosis or enterobacteria.
Results
The average departmental animal positivity rate is 5.145% (95% CI: 4.58 to 5.70), and at the herd level, 29.45% tested positive (95% CI: 27.69 to 31.22). The percentage of vaccinated heifer calves is 93.42% (95% CI: 93.36 to 92.47). The positive free herds account for 37.42% (95% CI: 35.30 to 39.53) of the total free herds. The strength of the association between bovine brucellosis-positive animals and the number of vaccinated heifer calves per year is 60% of the maximum possible, and the number of female cattle slaughtered per year is 54% of the maximum possible. Multiple linear regression between brucellosis-positive cattle and the number of vaccinated heifer calves and female cattle slaughtered per year by departments revealed significant moderate correlations ($p<0.001$). The association between brucellosis-positive cattle and human cases of brucellosis and Gram-negative enterobacteria shows that the strength of the association is 17% and 20%, respectively, of the maximum possible, which is considered low but significant.
Conclusions
The use of the B19 strain is associated with serological positivity in the tests used in Colombia. This relationship could be further enhanced by the combined use of RB51 in adult cattle. The testing and slaughter scheme should be cautiously applied when using a smooth strain on a mandatory and massive scale without unequivocal records of animal age identification and registration (J.L. Saenz Llorente, personal communication, June 20, 2022). In ecological studies, given the group-level unit of analysis, it is not possible for the found correlations to be maintained at the individual level.

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1081 - Etiology of Severe Clinical Mastitis in Dairy Cows: Insights from French Veterinary Clinics

Author: Olivier SALAT, Luc DUREL , Guillaume LEMAIRE, Florent PERROT

Objectives

Clinical mastitis (CM) in dairy cattle is a costly production disease and the leading cause of antimicrobial use. It classifies itself into three categories: mild, moderate, and severe. Severe CM accounts for 9 - 21% of cases and has severe consequences, including pain, reduced milk production, culling, and even death. In severe CM, the presence of Gram-negative bacteria is more common. Severe CM can progress rapidly and lead to systemic inflammatory shock. Severe cases with generalized signs are medical emergencies and should immediately be treated. Although parenteral application of antibiotics, including critically important antimicrobials, is beneficial in treating sepsis that frequently occurs in cows with severe CM, knowing the dominant pathogen causing the infection can help determine the appropriate antibiotic for immediate treatment. This study focused on severe clinical mastitis and aimed to identify the predominant bacteria responsible for severe CM in France.

Material and methods

Nineteen veterinary clinics from the leading French dairy regions participated in this study. On the dairy producer's call, local veterinarians and occasionally farm staff took milk samples; the team had received prior training to collect milk aseptically. Veterinarians assessed clinical severity using a dedicated clinical report form after clinical examination. According to local denominations, this study included CM cases named "severe mastitis," grade-3 mastitis, or "acute mastitis with general symptoms". Trained Veterinary surgeons enrolled in this study to perform in-clinic standardized microbiological diagnostic tests per the recommendation of the *National Mastitis Council*. Briefly, researchers plated thirty microliters of milk sample onto a series of media (30µL per single plate) with a sterile calibrated (5% sheep blood agar and other selective media). Plates were incubated at 37°C under aerobic conditions and read at 12, 24, and 48 h. Organisms were enumerated and identified using laboratory techniques described elsewhere (catalase, aesculin hydrolysis, Gram staining, Lancefield test). Researchers attempted to properly differentiate *Escherichia coli* from other Gram-neg species to prevent mistakes. Only the bacteriological data of clinically severe cases was in consideration for the analysis.

Results

Researchers examined 2087 severe clinical mastitis cases across the nineteen clinics; the number of reported cases varied greatly per clinic, ranging from 15 to 464. To prevent bias in the dataset, researchers randomly limited the number of records to 100 per clinic for those with high numbers and the calculation of prevalence accordingly. Concerning the participating vet clinic, Gram-negative bacteria were isolated in 61.3% of cases (95%CI[58.6;64.0]). *E. coli* was the most common pathogen, found in 53.9% of cases (95%CI[51.1;56.7]), followed by *Streptococcus Uberis* in 10.8% (95%CI[9.9;11.7]), and *Staphylococcus aureus* in 8.0% (95%CI[6.5;9.5]) of cases.

Conclusions

While relatively rare, severe clinical mastitis has significant economic and animal welfare implications. This study confirms the relevance of in-house microbiological analysis and

its application to the rational choice of antimicrobial treatment for severe mastitis. This study in major French dairy regions identified *E. coli* as the primary causative agent, emphasizing the importance of early and targeted antibiotic therapy, particularly against coliforms. Dairy producers and veterinarians should classify the severity of mastitis to guide effective treatment and prevent complications. This information is valuable for practitioners managing severe clinical mastitis in dairy cows.

1092 - Antimicrobial susceptibility of mastitis bacterial pathogens isolated from North American dairy cattle, 2011-2022

Author: Michael Sweeney , Lacie Gunnett, Dipu Mohan Kumar, Bryce L. Lunt, Michele Barrett, Abhijit Gurjar, Elizabeth Doré, Juan R. Pedraza, Véronique Moulin

Objectives
To determine the *in vitro* activity of ceftiofur, pirlimycin, penicillin-novobiocin, cefoperazone, ampicillin, erythromycin, oxacillin, and cephalothin against bacterial pathogens from naturally occurring cases of bovine mastitis in the United States and Canada.
Material and methods
A total of 10,890 bacterial isolates of *Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus,* and *Escherichia coli* isolated as etiological agents from dairy cows with mastitis by 29 veterinary laboratories across North America between 2011 and 2022 were tested for *in vitro* antimicrobial susceptibility by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) standards.
Results
Using available clinical breakpoints, antimicrobial resistance among *S. dysgalactiae* (n=2406) was low for penicillin-novobiocin (0% resistance), ceftiofur (0.1%), erythromycin (3.2%), and pirlimycin (4.6%). Among *S. uberis* (n=2398), resistance was low for ampicillin (0%) and ceftiofur (0.2%) and moderate for erythromycin (11.9%) and pirlimycin (18.4%). For *S. aureus* (n=3194), resistance was low for penicillin-novobiocin (0%), ceftiofur (0.1%), oxacillin (0.2%), erythromycin (0.7%), cefoperazone (1.2%), and pirlimycin (2.8%). For *E. coli* (n=2892), resistance was low for ceftiofur (2.8%) and cefoperazone (3.4%) and moderate for ampicillin (9.2%).
Conclusions
Mastitis pathogens in the United States and Canada have not shown any substantial changes in the *in vitro* susceptibility to antimicrobial drugs used for mastitis management over the 12 years of the study, or among that of a previous survey from 2002-2010. The data supports the conclusion that bacterial resistance to common antimicrobial drugs among mastitis pathogens, even to drugs that have been used on dairies for mastitis management for many years, continues to remain low.

1098 - Coagulase-negative Staphylococci as a cause of mastitis in ruminants: current knowledge and future perspectives.

Author: Rocio Angelica Ruiz Romero, Einar Vargas-Bello-Pérez

Objectives

Non-*aureus* staphylococci and mammaliicocci (NASM) are one of the most common causes of subclinical mastitis in dairy animals and the extent of damage of intramammary infections (IMI) caused by NASM is still under debate. The objective of this review is to provide a general background of the NASM as a cause of mastitis and the most recent advances that exist to prevent and fight the biofilm formation of this group of bacteria, introduce the new biomedical applications that could be used in dairy herds to reduce the risk of chronic and recurrent infections, potentially responsible for economic losses due to reduced milk production and quality.

Material and methods

Our search for information focused on studies reporting mastitis in ruminants caused by NASM and advances in the prevention of biofilms formation. A database was created from studies specifying the following topics: non-*aureus* staphylococci and mammaliicocci as a cause of mastitis in domestic ruminants, phenotypic and genotypic identification, pathogenesis, virulence factors, antimicrobial resistance, biofilm formation and biofilms treatment, covered the years 2000-2022, the search was conducted between July 2022 to October 2022.

Results

Non-*aureus* staphylococci and mammaliicocci (NASM) are part of a large group of Gram-positive bacteria that share their mutual lack of the coagulase virulence factor. Staphylococci are part of the normal skin flora of animals and have been isolated from different body sites from cows like hair coat, nares, teat skin, teat canal and from the dairy environment such as bedding as well as on the milker´s hands. In recent years, this group have become one of the main pathogens causing mastitis in domestic ruminants. The spread and prevalence of mastitis caused by NASM varies from one country to another, due to season dynamics and weather changes that affect the proportion of NASM pathogens that are spread among herds.

Biofilms are communities of microorganisms, which are bacteria that live naturally and preferentially as communities adhered to an inert surface or in living tissues, once adhered, the bacterial cells establish and organize themselves within a self-produced extracellular polymeric substance to form a matrix that provides protection against environmental threats, thus providing an extremely effective survival strategy. Biofilm is an important virulence factor in mastitis and as a result, infectious becomes more difficult to treat and eradicate, in fact, in dairy animals with mastitis, biofilms on the udders reduce the effect of antibiotics and allow microorganisms to evade the innate immune system.

Biofilm is one of the main causes of developing chronic infections, biofilm development is a complex process that involves many staphylococcal proteins that can be divided into

three general stages: attachment, multiplication/maturation, and shedding/dispersion. The new alternatives to prevent, treat and eradicate the biofilm and the bacteria found within it, have been used mainly in human medicine, while for veterinary medicine, the information on new biotechnologies to prevent the formation of biofilms in SCN is scarce. These alternatives can be adapted to dairy farms and monitor herds to be able to reduce the incidence of mastitis caused by CNS and avoid chronic infections due to the presence of biofilms. To prevent the development of biofilms, silver and copper ions can be used, surface modification using nanoparticles like zinc-oxide, silver or polyethyleneimine added to composite material and prevent bacterial growth. These positively charged metal ions adhere to the negatively charged bacterial cell wall and cause cell lysis and death. There is an urgent need for long-term active coatings that work against multiple bacterial strains and drug-resistant strains.

Conclusions

Since the pathogenicity of NASM is not entirely clear, animal models represent an alternative for the study of these pathogenic bacteria. Formation of biofilms must be avoided by adapting technologies that are currently used in human medicine, that have the potential to be used in dairy farms, where the different technology can be adapted to avoid the formation of these bacterial communities, which would be the main thing instead of facing established biofilm communities that are more difficult to eliminate.

1110 - An innovative veterinary vaccine adjuvant induces specific effector, memory and polyfunctionnal T-cells response

Author: Stephane Ascarateil , Laurent Dupuis

Objectives

Vaccine adjuvants are key components regarding vaccine formulation. The way they onset the immune response will determine the quality, intensity, and duration of the protection induced. Therefore it is essential to better understand the structure-to-function relations and the mechanisms of actions of the adjuvant. Here we present an in-depth immunological pattern analysis in the murine model of “Ictyolane 17” a new Water-in-Oil (W/O) commercially available veterinary adjuvant for cattle and sheep vaccines.

Material and methods

We have used a viral model protein in suboptimal concentration. The protein is CD8 pathway oriented but is sensitive to adjuvant switch. C57bl/6 black mice have been immunized subcutaneously twice, at day 0 and day 28 and slaughtered at day 42. Three groups were compared including antigen alone, antigen in Ictyolane 17, and antigen in reference adjuvant (not disclosed). Mice have been followed up for general safety and local reactions at injection site along the study. Blood have been collected for antibody response measurements at day 28 and day 42. Lung and spleen have been collected at slaughtering day. Cytokine production cells have been counted by Elispot and TCells polyfunctionality was checked by Fluorospot. Flow cytometry was used to determine the presence of resident cells in lung parenchyma and for the determination of memory Tcells phenotypes.

Results

“Ictyolane 17” induces an strong specific antibody response, compared to the antigen alone and to a similar commercial adjuvant, with a Th1/Th2 ratio oriented towards a Th1 response. It induces a high INFg response in the spleen and lung. We have evidenced the presence of polyfunctional cells capable of producing IFNg, IL2, and TNFa. “Ictyolane 17” has induced a high IFNg response on CD4+T and CD8+ T cells in the spleen and lung by ISC. We have found high levels of memory CD8+ T with a stronger stimulation of Effectory Memory T cells (TEM) than of Central Memory T cells (TCM) in the lung and spleen. “Ictyolane 17” also induces a high level of specific CD8+ T cells that were located within the lung parenchyma. In addition, there were no signs of local or general toxicity in this trial.

Conclusions

In this study we have used a viral protein in the murine model to analyse the immunological patterns of “Ictyolane 17” an innovative W/O adjuvant for bovine and ovine vaccines. We have found that “Ictyolane 17” could significantly enhance the immunogenicity of the antigen, inducing efficient cellular and humoral responses in a quantified and modulated safety profile.

1118 - Unexpected diarrhoea in adult cows can be caused by bovine coronavirus and needs proper feed management measures: a case report.

Author: Henk Kuijk, Bernd Hietberg, Frederik Waldeck, Geert Vertenten, Bart Sustronck

Objectives

Mandatory herd vaccination programmes are often hampered by the occurrence of suspected adverse events. However, a systematic approach of such pharmacovigilance cases is necessary to investigate the possible causal relationship between the applied vaccination and the observed clinical picture.

The present case report describes the systematic investigation of an outbreak of diarrhoea and milk drop on a Dutch Holstein-Friesian dairy farm shortly after mandatory whole herd vaccination against bovine herpesvirus 1 (BoHV1).

Material and methods

As part of the mandatory BoHV1 whole herd vaccination programme, in place since 2018, a six monthly booster vaccination was executed on a Dutch Holstein-Friesian dairy farm in January 2023. In total 200 milking cows were vaccinated with an inactivated gE deleted BoHV1 marker vaccine [Bovilis® IBR Marker Inac] and 110 young stock animals were vaccinated with a live, attenuated gE deleted BoHV1 marker vaccine [Bovilis® IBR Marker Live]. Three days after the whole herd vaccination the first cows with diarrhoea were observed. Approximately one week after the vaccination 30% of the cows presented diarrhoea and on average the milk production dropped from 27.5 kg to 22 kg. None of the young stock presented symptoms. Forage composition was unchanged since October 2022. Concentrate allowance was automatically adjusted to the production level in the robotic milking system. Forage intake remained constant during the outbreak. According to the farmer up to 90% of the cows showed a milk production decline which lasted for at least three weeks. The decreased milk production continued even after cessation of the diarrhoea and accounted for a total production loss of 12.000 kg of milk, according to the farmer. Paired sera were collected from nine affected cows for antibody determination against BoHV1, bovine viral diarrhoea virus (BVDV), Schmallenbergvirus (SBV), bovine coronavirus (BCoV) and Salmonella B/D LPS. However, the first blood samples could only be collected three weeks after the initiation of the outbreak due to logistic issues. Feed composition analysis with emphasis on phosphorus content was performed by the herd veterinarian. Five dairy cows were sampled for the determination of the serum concentration of trace minerals copper, zinc, iodine and selenium. Additionally, all animals older than 4 years (n=103) were screened for BoHV1 gE antibodies to evaluate the mandatory vaccination programme.

Results

No seroconversion was seen for BoHV1, BVDV, SBV and Salmonella B/D LPS. In seven out of nine cows high titers against BCoV were observed in the first serum sample. The two other cows showed a clear seroconversion for BCoV. Feed analysis indicated a low phosphorus content (< 4 gr phosphorus/ kg dry matter). Serum selenium levels in the cows were low (on average 0.4 µmol/l). Fifty out of 103 cows were seropositive for BoHV1 gE antibodies. All the seropositive animals were older than 5.5 years.

The prolonged milk production dip was mainly due to the automatically adjusted concentrate allowance in the robotic milking system. After manual adjustment of the

concentrate allowance the average milk production returned to levels as seen before the outbreak.

Conclusions

This case report shows that although an adverse reaction related to the whole herd BoHV1 vaccination was suspected, BCoV was the cause of a diarrhoea outbreak and milk drop in adult dairy cows. Low phosphorus and selenium levels in the feed probably have contributed to the severity of the problem on this particular farm since both phosphorus and selenium are involved in the resistance of cows against pathogen infections. In case of a disease outbreak on farms where the concentrate allowance is directly linked to the milk production level of the cows, it is recommended to adjust the concentrate allowance manually to avoid prolonged production losses after cessation of the clinical symptoms. The mandatory systematic BoHV1 whole herd vaccination programme, as implemented on this farm since 2018, seems to have worked since the BoHV1 gE positive animals on the farm are all born in or before 2017. This pattern indicates an old BoHV1 infection that is present in these cows and there has not been any recent circulation of virus in younger age cohorts.

1128 - Prospective cohort study in Dutch Holstein-Friesian female breeding calves to evaluate the evolution of Pasteurella multocida serum antibodies in the first six months of life.

Author: Henk Kuijk, Adriaan Beulens, Pleun Penterman, Hanny Swam, Geert Vertenten, Bart Sustronck

Objectives

Serologic BRD screening to identify which major respiratory pathogens are circulating on a farm (BRD quick scan) is frequently being used on dairy farms in The Netherlands to guide practitioners and farmers in the implementation of improved BRD farm management routines and to adjust and fine-tune BRD vaccination programmes (Kuijk et al 2022). *Pasteurella multocida* is very frequently detected in upper and lower respiratory tract samples in calves with BRD, necessitating the inclusion of *Pasteurella multocida* in the routine BRD quick scan. In order to correctly interpret the result of a serologic BRD screening, including *Pasteurella multocida* antibodies, a better knowledge of the evolution of maternal *Pasteurella multocida* antibodies over time seems appropriate. The aim of the present study is to obtain better insight in the evolution of maternal *Pasteurella multocida* antibodies in the first 6 months of life of Dutch Holstein-Friesian female breeding calves.

Material and methods

A prospective cohort study was conducted on a convenience sample of 5 Dutch Holstein-Friesian dairy herds, all belonging to the same veterinary practice. Inclusion criterion for the farms was a farm size of at least 100 milking cows. On each farm five new-born calves were randomly selected to be included in the trial. All calves received at least 3 litres of colostrum from their own dam in the first 24h after birth. The calves were blood sampled in the first month of life and each month thereafter for 6 consecutive months. The serum samples were analysed in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands) for antibodies against *Pasteurella multocida* by ELISA using an in-house test. Data concerning occurrence of calf disease were obtained from the herd record keeping logbooks. The evolution of the *Pasteurella multocida* antibodies over time was visually assessed. The association of calf disease occurrence with calf *Pasteurella multocida* antibodies was explored using generalized linear mixed models including farm as random effect. Statistical analysis was conducted in R (R Core Team 2022).

Results

This longitudinal study is ongoing. The preliminary data show that maternal antibodies against *Pasteurella multocida* sharply decline during the first month of life but can still be detected in Dutch Holstein-Friesian female breeding calves at that time. Full results of this prospective cohort study will be presented at the congress.

Conclusions

This prospective cohort study revealed new insights into the evolution of maternal antibodies against *Pasteurella multocida* in Dutch Holstein-Friesian female breeding calves. The findings of this study will contribute to an objective and correct interpretation of the results of serologic BRD screenings (BRD quick scan) on Holstein-Friesian dairy farms.

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1140 - Utilization of nanoplate digital PCR for the quantification of ovine gammaherpesvirus 2 in subclinically infected animals

Author: Selwyn Headley Arlington, Luara Evangelista Silva, Isabela Vaz Silva, Maria Constanza Rodriguez

Objectives

Ovine gammaherpesvirus 2 (OvGHV2) is the cause of sheep associated-malignant catarrhal fever (SA-MCF), a severely fatal lymphotropic disease that affects a wide variety of mammalian hosts. Infections by OvGHV2 produces results in several well-defined clinical syndromes that are readily identified by practicing veterinarians. Animals with these clinical syndromes generally have elevated viral loads of OvGHV2. However, dead-end mammalian hosts infected with OvGHV2 are frequently subclinically infected without the demonstration of the classical syndromes of SA-MCF. This report describes the utilization of nanoplate digital PCR (dPCR) methodology for the detection of extremely low viral loads of OvGHV2 in animals subclinically infected.

Material and methods

The tegument protein gene of OvGHV2 derived from animals infected with OvGHV2 were amplified and used as inputs for the dPCR assay. Clinical samples were derived from a cow with the head and eye form of SA-MCF (Animal #1); a cow with the neurological form of SA-MCF (Animal #2), urine from an asymptomatic cow (Animal #3), and a subclinically infected goat (Animal #4). These samples were compared with a pool of positive controls and a synthetic DNA of OvGHV2. The dPCR assay consisted of 0.1 µM of primer forward and reverse primers, and 0.2 µM of the hydrolysis probe designed to amplify specific 80 base pairs (bp) of the OvGHV2 DNA polymerase gene. The dPCR assays were carried out in single-plicate with 10 µL of DNA, added to 10 µL of Probe PCR Master Mix (Qiagen, Hilden, Germany) and 26000 24-well Nanoplates (Qiagen, Hilden, Germany), to the 0.8 µM forward, 0.8 µM reverse primers and 0.4 µM probe, in a final volume of 40 µL. The nanoplate was then loaded onto the QIAcuity One dPCR (Qiagen, Hilden, Germany). The quantification cycle protocol consisted of 95 °C for 2 min for enzyme activation, 95 °C for 15 s for denaturation, and 60 °C for 30 s for annealing/extension for 40 cycles, with a final imaging step made by reading in the FAM channel. The absolute number of viral DNA sequences was determined by dPCR using the QIAcuity One dPCR platform (Qiagen, Hilden, Germany) with field samples and synthetic OvGHV2 DNA. Absorbance measurements at 260 nanometers (nm) by UV spectrophotometry in a NanoDrop ND-ONE-W spectrophotometer (ThermoScientific, Waltham, MA, USA) and fluorescence measure with PicoGreen dye in a Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA, USA) were used according to the manufacturer's protocols to quantify DNA and the amounts of synthetic DNA molecule gBlocks® gene fragments (Integrated DNA Technologies, Coralville, IA, USA) of the 10-fold serial dilutions increments (Pure to 10^{-6}). The efficiency of the dPCR assay was then compared with a standard curve derived from a qPCR assay designed to amplify the same partial fragment of the OvGHV2 DNA polymerase gene.

Results

There was a direct relationship between the viral load (copy number/uL) determined by dPCR detection and the standard curve of the qPCR assay, i.e., samples with elevated viral load had a corresponding reduced quantification cycle (*Cq*). Elevated copies of viral DNA were detected in Animals #1 (14,400/uL) and # 2 (13,444/uL) with clinical manifestations of SA-MCF as well as the positive control (81,800/uL) and the synthetic DNA (1,4228/uL). Alternatively, comparatively reduced viral loads with consequent elevated *Cq* were identified in the goat (0.84/uL) and urine (1.28/uL) from a cow that were subclinically infected by OvGHV2. Furthermore, the standard curve generated from the qPCR assay demonstrated an amplification efficiency of 95.214%, correlation coefficient (R2) of 0.998, and a slope of − 3.44.

Conclusions

The dPCR assay was efficient in the detection of viral loads of OvGHV2 in animals with and without clinical manifestations of SA-MCF, demonstrating that this novel PCR assay can be used for the detection of OvGHV2 DNA even in animal without clinical manifestations of SA-MCF. These results demonstrated that there is a definite relationship between the OvGHV2 viral load in affected animals and the demonstration of clinical SA-MCF with typical histopathological findings. Furthermore, the low viral load in the goat may indicate that elevated viral loads are required to produce clinical manifestations of SA-MCF in this ruminant species. While the detection of OvGHV2 in the urine of an asymptomatic cow may suggest a possible via of environmental contamination.

1143 - Molecular and histochemical detection of Macavirus in aborted bovine fetuses

Author: Flávia Helena Pereira Silva, Juliana Torres Tomazi Fritzen, Julia Raisa Ximenes Figueiredo, Rafaela Maria Boson Jurkevicz, Ana Flávia Ferrreira Domingues, Milena Patzer Rose, Luara Evangelista Silva, João Luis Garcia, Amauri Alcindo Alfieri, Selwyn Arlington Headley

Objectives

Ovine gammaherpesvirus 2 (OvGHV2) is a member of the *Macavirus* genus and the cause of sheep-associated malignant catarrhal fever (SA-MCF) in a wide range of dead-end mammalian hosts. SA-MCF is a severe lymphotropic, frequently fatal disease of sporadic occurrence with low morbidity an elevated lethality. Macaviruses associated with the development of malignant catarrhal fever (MCF) share the 15A epitope and are referred to as malignant catarrhal fever virus (MCFV). There are few documented reports demonstrating the possible association between OvGHV2 and ruminant abortion. While the capacity of *Macavirus* to induce abortions in ruminants is currently unknown. This report presents the pathological and molecular findings observed in aborted bovine fetuses from Southern Brazil that were infected by *Macavirus*.

Material and methods

Four aborted bovine fetuses from three farms, located in Paraná State, Southern Brazil were received for pathological and molecular diagnosis. All farms had reported histories of repeated abortions. All fetuses were subjected to routine post-mortem evaluations and tissue fragments (liver, small intestine, thymus, myocardium, lungs, and kidneys) were routinely processed for histopathological evaluations. Selected formalin fixed-paraffin embeded (FFPE) tissue fragments of the previously mentioned organs from each fetus were used in immunohistochemical (IHC) assays designed to amplify tissue antigens of MCFV by using the 15A-monoclonal antibody (15A-MAb). Duplicate sections of the tissues used for histopathology, collected freshly were used in molecular assays designed to amplify nucleic acids of fetopathic agents of cattle, including bovine viral diarrhea virus (BVDV), bovine alphaherpesvirus 1 (BoAHV1), *Histophilus somni*, *Neospora caninum*, *Listeria monocytogenes*, and *Leptospira* spp. Additionally, molecular assays were done to identify two *Macavirus* previously diagnosed in ruminants, OvGHV2 and bovine gammaherpesvirus 6 (BoGHV6).

Results

The ages of the fetuses as determined by the crown and rump distance, varied between 78 to 208 days. Significant pathological alterations were not observed in any of these fetuses. The 15A-MAb IHC detected MCFV antigens in the liver, lungs, kidneys, and thymus from two fetuses (#1 and #4); positive immunoreactivity to the 15A-MAb IHC assay was not identified in Fetus #2 and #3. Molecular testing amplified OvGHV2 DNA only from the myocardium and lungs of Fetus #1 that had positive intracytoplasmic immunoreactivity to the 15A-MAb IHC assay in multiple tissues. Furthermore, the *Leptospira* spp. PCR assay confirmed infections in Fetuses #1, #3, and #4, while PCR detected *Neospora caninum* in the myocardium of Fetus #2. Additionally, the nucleic acids of BVDV, BoAHV1, BoGHV6, *H. somni*, and *L. monocytogenes* were not detected in any of the tissue evaluated.

Conclusions

These findings confirmed that the 15A-MAb IHC assay can be efficiently used to detect MCFV antigens in tissues of aborted bovine fetuses. We have previously used this IHC assay for the detection of MCFV antigens in cattle and sheep infected with OvGHV2. The identification of MCFV antigens with the simultaneous detection of OvGHV2 DNA confirmed that Fetus #1 was infected by OvGHV2 and adds to the few descriptions of this infection in aborted fetuses of ruminants worldwide. Furthermore, the IHC detection of MCFV in multiple organs of Fetus #4, without the molecular detection of OvGHV2 or BoGHV6, suggesting that this fetus was probably infected by a *Macavirus* that was not previously diagnosed in cattle from Brazil. The identification of Leptospira spp and N. caninum in these fetuses suggest that these fetopathic agents were associated with the reproductive problems at these farms. Alternatively, the non-amplification of BVDV, BoAHV1, BoGHV6, *H. somni*, and *L. monocytogenes* suggest that these fetopathic agents were not associated with the repeated abortions at these farms. Although OvGHV2 was identified in one of these fetuses, additional studies must be done to determine the possible effect of this virus on the development of fetal disease.

1147 - Isolation and Identification of bacteria causing mastitis in dairy cows and their susceptibility to antibiotics in Aguascalientes, Aguascalientes, Mexico

Author: Valeria Alejandra Morales-Murillo, Rocio Angelica Ruiz Romero, Luis Armando Contreras-Méndez, Gretel Iliana Gil-González, Einar Vargas-Bello-Pérez

Objectives

Mastitis is defined as inflammation of the mammary glands that can be caused by trauma or infectious agents, such as bacteria, fungi and viruses; of these aetiologies, bacterial mastitis is the most common. This disease affects the physical, chemical and bacteriological characteristics of milk. Clinical mastitis is characterized by pathological changes in the mammary gland, such as inflammation, loss of the ability to produce milk and alterations in the color and consistency of the milk. In the case of subclinical mastitis, there are no visible signs of disease since the milk exhibits a normal appearance. Accordingly, this disease may only be detected through the use of diagnostic techniques based on the quantification of somatic cells in the milk. Among the most common bacteria that may cause mastitis are Staphylococcus spp., Streptococcus spp., enterobacteria and Mycoplasma spp. The most commonly identified genus is Staphylococcus, within which the most important species are Staphylococcus aureus and coagulase-negative staphylococci (CoNS).

The aim of this work was to isolate bacteria causing clinical and subclinical mastitis in milk from Holstein cows and milking equipment in a barn located in Aguascalientes, Mexico to identify bacteria causing this disease, as well as the susceptibility pattern to Beta-lactams, macrolides and tetracyclines through phenotypic tests.

Material and methods

This work was carried out in a barn located in Aguascalientes, Mexico, with a total of 13 cows in production, the California Mastitis Test was performed to diagnose subclinical mastitis, milk samples were taken from the quarters positive to the test, the teats were disinfected with a swab and alcohol, 50 mL of milk was obtained from each of the affected glands and collected in new, sterile and airtight bottles, milk samples were frozen. Six sets of liners (24 samples in total) were also sampled using sterile swabs and transported in Stuart medium at 4ªC until processing in the laboratory.

In order to identify bacteria in milk, the primary culture was carried out on blood agar and McConkey agar plates using 30 µl of the sample. Both plates were incubated at 37 °C for 24 hours under aerobic conditions. The cultures were examined to determine the macroscopic morphology of the grown colonies. From the representative colonies, a Gram-stained smear was made for the biochemical identification recommended by Carter (1984).

To perform the chemotherapeutic susceptibility test, the agar diffusion method (also known as the Kirby-Bauer test) was performed.

Results

A total of 49 milk samples were obtained, 11 samples (22.45%) were positive as follows, 7/49 samples were positive for coagulase negative Staphylococci, 2/49 were positive for coagulase negative Staphylococci and *Corynebacterium* spp, 2/49 were positive for

coagulase negative Staphylococci and *Esherichia coli*, lastly 38/49 (77.55%) samples were negative for bacterial growth.
Regarding the susceptibility test to antibiotics it was only performed on isolates of *Staphylococcus* spp, 100% of the isolates were sensitive to ampicillin, erythromycin and tetracyclines, 27.27% were resistant to oxacillin and 0.9% were resistant to penicillin.

Conclusions

Attention should be paid to the handling and maintenance of the milking equipment, as mastitis may occur due to overmilking, poor pulsation and pressure calibrations, and inadequate washing of milking equipment. In both mechanical and manual milking, hygiene is the main prevention strategy for bacterial mastitis. it is important to carry out studies to detect resistance genes to antibiotics, as the species identified show resistance to the antibiotics used in the antibiograms of this study.

1149 - An epidemiological study on dairy farms in Europe evaluating prevalence, biosecurity and management associations with bovine coronavirus infections indicates an endemic disease situation.

Author: Anna Catharina Berge , Geert Vertenten

Objectives
The objectives of this study are to obtain an estimate for the farm prevalence of Bovine Coronavirus (BCoV) in dairy production in Europe, and to characterize farm-level risk factors in management and biosecurity that are linked to BCoV infection in neonatal and weaned dairy calves.
Material and methods
This is a cross-sectional field study of 125 dairies in Europe, based upon a convenience selection of farms. Conventional dairy farms were visited at least once for sample collection and herd information. On every farm, one bulk tank milk (BTM) sample, 10-20 samples from neonatal calves, 10-20 samples recently weaned calves and 5-10 samples from fresh cows were collected. Nasal and faecal swabs were collected for BCoV detection using semi-quantitative Real Time PCR (RT-PCR), blood and BTM were collected for BCoV antibody detection using ELISA. An extensive questionnaire was performed to determine husbandry and management factors. Biosecurity was scored using the Biocheck (https://biocheckgent.com/). Data was entered analysed with SAS version 9.4. Multiple logistic regression models (binary and ordinal) with random effects of herds were used to evaluate relationship between husbandry, management, and biosecurity and BCoV on dairies.
Results
The study is ongoing, and we have analysed results from 125 dairy farms in Austria, Belgium, Czech Republic, Denmark, France, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Romania, Sweden, Slovakia and Scotland (United Kingdom). Antibody levels in BTM were on average 89 (++++). Mean antibody levels were 58 (++) in preweaned calves, 42 (++) in weaned calves and 65 (+++) in fresh cows. There was poor correlation (r=0.38) between BTM and mean serum antibody levels. Presence of BCoV was confirmed in 17% of nasal and faecal swabs with no significant difference in BCoV recovery from nasal versus faecal swabs. Virus was isolated in 80% of herds. Coronavirus was detected in 24% of samples from neonatal calves, 23% of samples from weaned calves, and 5% of samples from fresh cows. The biosecurity scored on 100 dairies had an overall Biocheck score of 60% (external score 71%, internal score 47%). Preliminary analysis indicates that virus shedding may be lower in herds with higher biosecurity scores in transport of animals and visitors. In herds where dams are vaccinated against BCoV in the dry period, the neonatal calves were shedding significantly less virus, however the weaned calves were shedding more virus. Further farm management and biosecurity risk factors for BCoV in dairy calves will be presented.
Conclusions
BCoV is commonly present in both the respiratory and enteric pathway in neonatal calves, weaned calves and fresh cows in EU dairies, with all study herds being seropositive to the virus, and the virus present in 80% of study herds. BCoV dry cow vaccinations are protective against neonatal calf infections, but not against weaned calf infections.

1153 - Prevalence of Q fever in dairy cattle farms with reproductive disorders

Author: Philippe Gisbert, Carla Azevedo, Juan Munoz-Bielsa, Pierre Charollais, Michaël Treilles

Objectives

Q fever is a bacterial disease present throughout the world (except New Zealand). It is caused by *Coxiella burnetii,* a small, gram-negative, intracellular bacterium. *Coxiella burnetii* can infect numerous animal species and is zoonotic. Among animals, ruminants are the most affected and are the reservoir of this pathogen. In small ruminants, particularly goats, clinical signs are dominated by abortions. In cattle, beside abortion, other clinical signs such as metritis, placental retentions and fertility disorders are also encountered.

The aim of this study was to determine the proportion of *Coxiella burnetii*-infected dairy cattle herds among those reporting abnormally high rates of reproductive problems.

Material and methods

PCR analysis of bulk tank milk (BTM) is a simple and affordable way of testing for *Coxiella burnetii* in dairy herds. In 2020, Ceva and Qualyse developed QTest to facilitate sample collection and transport to the laboratory. QTest combines an easy sampling medium (FTA card®) that inactivates micro-organisms while preserving nucleic acids, with a real-time PCR analysis carried out in a single laboratory in France. The use of QTest increases the sensitivity of BTM analysis[1].

With every Qtest, a sample identification form is also included. On the form, the breeder or veterinarian who sends the sample reports any reproductive disorders present on the farm. It is possible to tick more than one box:

- Abortions / premature delivery / stillbirth / weak born offspring (APSW complex)
- Metritis / endometritis
- Retained placenta
- Infertility

The aim of QTest is to identify whether *Coxiella burnetii* is present in herds experiencing reproductive disorders. For the present study, we have analyzed the results obtained during a period of 3 years from October 2020 to September 2023. Herds were classified in two groups. The first group was made of herds that had reported the APSW complex and the second group was made of herds that had reported any of the other reproductive disorders. As it was possible to report several clinical signs, it was decided to assign to the APSW complex group, herds where both APSW complex and at least one other clinical sign were reported.

Results

During the study period 1091 samples were collected from farms where at least one reproductive disorder had been reported. The geographical origin is as follows:

- France n=332
- Italy n=273
- United Kingdom n=261

- Germany n=142
- Greece n=20
- Republic of Ireland n=17
- Belgium n=16
- Romania n=15
- Poland n=6
- The Netherlands n=3
- Tunisia n=3
- Turkey n=2
- Bulgaria n=1

APSW complex was reported on 619 farms (56.7%). The presence of *Coxiella burnetii* in BTM was detected in 41.8% of them (n = 259). 472 farms (43.3%) reported other reproductive disorders. Of these, 42.8% were positive for *Coxiella burnetii* in BTM (n = 202). The difference in prevalence was not statistically different between the two categories (p=0.752, Chi-square test). These results suggest that the presence of *Coxiella burnetii* in dairy cattle can be associated without difference with major reproductive disorders (abortions, premature delivery, stillbirth, weak born calves) or other reproductive disorders (metritis/endometritis, retained placenta, infertility).
For the four countries for which we received the most samples, *Coxiella burnetii* was detected in 39.8%, 42.5%, 51.0% and 32.4% of samples from France, Italy, the UK and Germany, respectively. In a previous study[1], we analyzed 414 BTM samples from randomly selected farms (with and without reproductive disorders) in France. 16.9% were positive (n = 70). By comparing with the results of the present study for France, farms reporting reproduction disorders were 3.24 times more likely to be infected with *Coxiella burnetii* (95% CI: 2.32; 4.55).

Conclusions

Our study showed that in around 42% of dairy farms with reproductive disorders (abortions, premature delivery, stillbirth, weak born calves, metritis, endometritis, retained placenta or infertility), *Coxiella burnetii* was present in bulk tank milk. This study also showed that Q fever can run in a dairy farm without abortion cases but causing other reproductive disorders. Dairy herds with any of the reproductive disorders evaluated in this study should include Q fever in their differential diagnosis.

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1155 - Cross-neutralization between Bovine Viral Diarrhea Virus (BVDV) types 1 and 2 after vaccination with a BVDv-1a modified-live-vaccine

Author: Philippe Gisbert, Géromine Grange, Marie Mindeguia, Gilles Meyer

Objectives

BVDV-1 and BVDV-2 are responsible for major reproductive problems in cattle such as abortions, pregnancy losses and, most importantly, the birth of Persistent Infected (PI) animals that are responsible for maintaining the disease in the herd. This results in large economic losses.

Since there are many genotypes within BVDV-1 and BVDV-2 species, it is important to know whether the use of a monovalent vaccine could protect cattle against other genotypes. This study provides evidence that serum from animals immunized with Mucosiffa®, a modified-live-vaccine containing a BVDV-1a strain can neutralize other BVD-1 and BVDV-2 strains in-vitro.

Material and methods

A study to obtain the "12-month fetal protection" indication for Mucosiffa® was conducted. Fetal protection was demonstrated in an experimental challenge with the BVD-1f-Hanover strain inoculated 363 days after vaccination (Achard *et al.,* 2018). Seronegative heifers were vaccinated (D0) and blood samples were taken at D28, D203 and D363 before challenge.

For the present study, these sera were used to test their ability to neutralize several strains of BVDV: 1a-NADL, 1f-Hanover, 1e, 1b and two BVD-2a strains. All these strains are non-cytopathic except the BVDV-1a-NADL.

Seroneutralization (SN) assays were performed for the 6 BVDV strains and each selected day in a 96-well cell microplate (4 duplicates per sample) with a constant amount of virus (200 TCID50 per well) as already described (Hamers *et al.,* 2002; Meyer *et al.*, 2021). The reading was taken after staining and the titers were calculated as the inverse of the serum dilution protecting 50% of the cell culture wells. They were expressed as Log2ED50/mL using the Spearman-Kärber method.

A two-way ANOVA with repeated measures (three-factor split-plot ANOVA) was used to analyze SN titers. When the effects of the “day” and “treatment” factors were significant among interactions, a Bonferroni test between contrasts was used to compare the treatments on each day post challenge.

Results

We identified three different patterns of virus neutralizing titers (VNT), depending on the strains.

- BVDV-1a and BVDV-1b: rapid increase until D28 followed by a lower increase until D203 and then stability in time (until D363)
- BVDV-1e and BVDV-1f-Hanover: rapid increase until D28, stability until D203 and then slightly decrease until D363
- BVDV-2a: increase until D203 and then stability until D363. At D28, VNT were statistically lower than for the BVDV-1 strains.

Interestingly, statistical analysis showed no significant difference ($p>0.05$) in VNT between BVDV-1f-Hanover, BVDV-1e and the two BVD-2 strains at D203 and D363. As

the vaccine has been shown to be effective to prevent a fetal contamination with the BVDV-1f-Hanover strain, 363 days after vaccination, this suggests that Mucosiffa® vaccination provides humoral cross immunity, which may also protect against fetal infection by BVDV genotype 2 (Grange *et al.,* 2023).

Conclusions

Our study showed that sera from cattle vaccinated with Mucosiffa® were able to neutralize strains of BVDV-2a. Interestingly, neutralizing antibody titers against these BVDV-2a strains and the BVDV-1f-Hanover strain were similar from D203. Although confirmation by experimental challenge with a BVDV-2a strain could be useful, we can hypothesize that the vaccine is clinically effective against infection with a BVDV-2a strain.

References

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1158 - Field Safety of a modified live attenuated Mycoplasma bovis vaccine in young calves

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Objectives

Mycoplasma bovis is an important pathogen in the occurrence of respiratory disease in young calves. Two studies were performed to evaluate the safety of a modified live attenuated *Mycoplasma bovis* vaccine [Protivity] in young calves under field conditions.

Material and methods

The study was conducted under the supervision of the Zoetis Institutional Animal Care and Use Committee (IACUC). In Study 1, 1069 conventionally raised calves from three geographical regions in the United States were randomly allocated to a non-vaccinated T01 control group (n=355) or one of two groups (T02 and T03) that were administered two subcutaneous doses of the modified live *M. bovis* vaccine (total calves dosed n=714) at an interval of 21 days. Of the vaccinated animals, about half were between 1 and 8 days old, while the other half were around 1 month old at first vaccination. The younger calves were the target age for the vaccine and were enrolled on an intensive calf rearing ranch. Animals were observed for local and systemic reactions, injection site reactions and daily general health post vaccination for any adverse event (AE). The cause of death of any animal that died or was euthanized was investigated by diagnostic pathology to establish any association to the vaccine. The study ended after 56 days. In Study 2, 148 calves between 4 and 7 days old were allocated to a non-vaccinated group (n=51) or a group that was administered two subcutaneous doses of the modified live *M. bovis* vaccine (n=97) and were observed daily for general health for a longer duration of time (120 days). Deep nasal swabs were collected every two weeks from each calf for the first 56 days of Study 2. Any adverse events and cause of death were fully investigated.

Results

In Study 1, no injection site reactions were observed and the incidence of adverse events (AEs)was similar with 25.6% observed in the unvaccinated animals and 22.85% (average of 19.7% T02 group and 26% T03 group) in the vaccinated group. Overall, there was no increase in any unexpected adverse events that could be attributed to the administration of two doses of the vaccine when given subcutaneously. Most of the AE were consistent with normal young calf health issues (enteric, respiratory and lameness). A total of 20 control and vaccinated animals had to be removed from the study due to unresolvable health events. There were 10 control calves and 10 vaccinated calves across the three geographical study locations that were removed from study, representing 2.8% (10/355 calves) and 1.4% (10/714 calves) from the controls and vaccinates, respectively. Of the total vaccinated calves, one out of 714 showed an AE that could potentially be attributed to the vaccine. In Study 2, a total of 32 adverse events occurred in 26 animals, with equal frequencies in both control and vaccinated groups. None of the AEs were considered to be related to the vaccine. *Mycoplasma bovis* was never recovered in any of the deep nasal swabs, confirming that the *M. bovis* vaccine strain was not shed from vaccinated animals.

Conclusions

The data demonstrated the safety of a two-dose *M. bovis* vaccine [Protivity] when administered subcutaneously to young cattle under field conditions.

1185 - Combined Effects of Two Bovine Antimicrobial Peptides on a Mannheimia haemolytica field isolate

Author: Santiago Cornejo Tonnelier, Keun Seok Seo, Mark Lawrence, Amelia Woolums

Objectives

Mannheimia haemolytica (Mh) is the principal bacterial pathogen associated with bovine respiratory disease (BRD) in cattle. Resistance to antimicrobials historically used for BRD is becoming prevalent; administration of antimicrobial peptides could improve treatment efficacy and slow the spread of antimicrobial resistance. We measured the minimum inhibitory concentration (MIC) of two bovine antimicrobial peptides, Bovine Myeloid Antimicrobial Peptide 28 (BMAP-28) and Bactenecin 5 (Bac-5), alone or in combination on a Mannheimia haemolytica (Mh) field strain.

Material and methods

Individual effects were established by MIC assays and the combined effects were established by calculation of the Fractional Inhibitory Concentration Index (FICi).

Mh field strain 35-248 was used in this study. Mh were individually plated on Mueller Hinton (MH) agar and incubated for 24hs at 37°C, 5% CO_2. After 24hs, a suspension at 0.5 McFarland standard was prepared, and two ten-fold dilutions were made in MH broth to reach the desired $1x10^6$CFU/ml inoculum concentration.

BMAP-28 and Bac-5 dilutions were prepared in MH broth, with concentrations ranging from 512 to 0.25µg/ml in a serial two-fold dilution pattern.

Using a 96 well plate, 50µl of the Mh inoculum were tested against 50µl of the peptides alone or in a series of combinations in a 1:1 ratio using a checkerboard assay. MH broth was plated for sterility and 100µl of the inoculum were used as the growth control.

The plate was incubated at 35+/-2°C in a shaker incubator for 20-24hs and MICs were determined. This experiment was repeated twice.

Results

The MICs of BMAP-28 and Bac-5 were 64µg/ml and 128µg/ml, respectively. The FiCi of the antimicrobial peptide combinations were <0.5 for all combinations tested, denoting a synergistic effect, and resulting in decreases of MICs of up to 256-fold for BMAP-28 (0.25µg/ml) and up to 16-fold for Bac-5 (8µg/ml).

Conclusions

Both antimicrobial peptides have a measurable effect on the Mh field strain tested and can be used in combination to lower the MIC of both peptides, with more impact to reduce the MIC of BMAP-28. Combined treatment with bovine antimicrobial peptides may help improve BRD treatment efficacy and potentially help delay the onset of resistance.

1191 - The prevalence, isolation, and molecular characterization of Dabie bandavirus from cattle in the Republic of Korea

Author: Joon-Seok Chae , Jeong-Byoung Chae

Objectives
Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by the *Dabie bandavirus*. First identified in China, the disease has now spread across various Asian countries, primarily through tick bites and animal-to-human transmission. This study focused on investigating SFTS virus (SFTSV) infections in cattle in the Republic of Korea (ROK). Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by the *Dabie bandavirus*. First identified in China, the disease has now spread across various Asian countries, primarily through tick bites and animal-to-human transmission. This study focused on investigating SFTS virus (SFTSV) infections in cattle in the Republic of Korea (ROK).
Material and methods
Over two years (2019 and 2020), 845 cattle serum samples in the ROK were collected. Viral RNA was extracted from these samples using a viral RNA extraction kit. One-step RT-nested polymerase chain reaction (PCR) was then employed to amplify the S segment of SFTSV. Positive serum samples facilitated the isolation of SFTSV in Vero E6 cells, where the virus underwent three culture cycles. Post-culturing, we conducted a comprehensive analysis of the full sequence of SFTSV. The sequencing data were processed using Chromas and aligned with Clustal X. We constructed a phylogenetic tree using the maximum-likelihood method in MEGA7.
Results
Out of the samples, 34 were positive for SFTSV S segments, and one virus isolate was successfully cultured in Vero E6 cells. Our phylogenetic analysis, based on the partial S segment, categorized four SFTSV isolates as B-2 genotype, nine as B-3 genotype, eighteen as B-4, and three as D genotype. Notably, one cultured virus was classified as B-2 genotype, based on the comprehensive analysis of the L, M, and S segments of SFTSV.
Conclusions
To our knowledge, this is the first successful isolation of SFTS virus from cattle in the ROK. Our findings underscore the need for more rigorous and continuous monitoring of SFTSV in cattle and other animals. Such surveillance is imperative to understand the virus's genetic diversity and assess its potential eco-epidemiological impact on human health.

1201 - Antimicrobial resistance phenotypes of Mannheimia haemolytica and Pasteurella multocida isolates from Bovine Respiratory Disease (BRD)-affected and healthy animals in feedlots in Spain

Author: Johan Manuel Calderón Bernal, Carlos Serna, Julio Álvarez, Ángel García Muñoz, Alberto Díez Guerrier , Lucas Domínguez, José Francisco Fernández-Garayzábal, Ana Isabel Vela, María Dolores Cid

Objectives

BRD is the primary cause of antimicrobial use (AMU) in beef cattle industry, both as metaphylaxis and as individual treatments, to control bacterial infections which are the ultimate responsible for pneumonic lesions and clinical signs of the disease. *M. haemolytica* and *P. multocida* are commensals of the bovine respiratory tract and major bacterial pathogens in BRD. Although Spain is one of the main beef cattle producers in the European Union, studies investigating the antimicrobial susceptibility of *M. haemolytica* and *P.* multocida are lacking. The aim of this study was to investigate and compare the antimicrobial susceptibility phenotypes of *M. haemolytica* and *P. multocida* isolated from both apparently healthy animals (AHA) at the time of entry into feedlots and those from BRD-affected animals (BRD-AA) during the fattening period in the same batches of the feedlot.

Material and methods

A total of 54 *M. haemolytica* and 75 *P. multocida* isolates from BRD-AA (*n*= 30 and n= 25, respectively) and AHA (*n*= 24 and n= 50, respectively), belonging to 17 and 15 different animal batches, respectively, housed in five feedlots located in Madrid and Valencia were subjected to antimicrobial susceptibility testing. Minimum inhibitory concentrations (MIC) for 19 antimicrobials were determined by broth microdilution method using the Sensititre BOPO7F Bovine/Porcine Panel. MIC50 and MIC90, which inhibit bacterial growth by 50 % and 90 % of isolates, respectively, were determined. Isolates were classified as non-wild type (NWT) using the epidemiological cut-offs (ECOFFS) of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) when available, or as non-susceptible (intermediate or resistant; NS) based on the clinical breakpoints according to Clinical and Laboratory Standards Institute (CLSI) guidelines or Comité de l'Antibiogramme of the French Society of Microbiology (FSM) when not. NWT and NS isolates were named as resistant from hereon.

Results

Resistance to 16 of the antimicrobials tested was detected among *M. haemolytica* isolates, including ampicillin (3.7%), penicillin (44.4%), gentamicin (18.5 %), neomycin (35.2 %), spectinomycin (35.2%), tildipirosin (37.0 %), tilmicosin (70.4%), tulathromycin (51.9 %), gamithromycin (53.7 %), tiamulin (1.9 %), enrofloxacin (38.9 %), danofloxacin (22.2 %), florfenicol (46.3 %), tetracycline (46.3 %), sulphadimethoxine (≥ 40.7%), and trimethoprim-sulfamethoxazole (1.9%). Among *P. multocida* isolates resistance was detected to 11 antimicrobial including neomycin (20.0 %), spectinomycin (10.7 %), tildipirosin (9.3 %), tulathromycin (29.3 %), gamithromycin (28.0 %), enrofloxacin (14.9 %), danofloxacin (17.3 %), florfenicol (5.3 %), tetracycline (32.0 %), sulphadimethoxine (≥ 73.3 %) and trimethoprim-sulfamethoxazole (29.1 %). The higher resistance levels found among isolates of both pathogens were to macrolides (tilmicosin, tulathromycin and gamithromycin for *M. haemolytica*, and tulathromycin and gamithromycin for *P.*

multocida), and tetracycline. Resistance levels were also high for penicillin and florfenicol among *M. haemolytica* isolates (44.4 % and 46.3 %, respectively). Both *M. haemolytica* and *P. multocida* isolates from BRD-AA showed resistance levels significantly higher ($P < 0.05$) than those from AHA for all the antimicrobials to which resistances were detected. Multiresistant phenotypes (resistance to three or more antimicrobial families) were detected in 50 % and 32 % of *M. haemolytica* and *P. multocida* isolates, respectively. The most frequent resistotypes detected in *M. haemolytica* isolates included resistances to aminoglycosides, macrolides, quinolones, phenicols, tetracyclines, in addition to sulfonamides (14.8%) or beta-lactams (13.0%). The most common resistoypes among *P. multocida* isolates included resistance to aminoglycosides, macrolides, tetracyclines and sulphonamides (13.3 %).

Conclusions

In agreement with previous studies in other countries, antimicrobial resistance was common both in *P. multocida* and *M. haemolytica* Spanish isolates, with elevated to high resistance levels to several antimicrobials commonly used to control BRD. Even though resistances were significantly higher among BRD-AA isolates, resistances were also detected among isolates from AHA at the entry to the feedlots, which underlines the need for implementing antimicrobial surveillance programs to control and reduce the spread of antimicrobial resistances in these pathogens within and between herds. Due to the differences in antimicrobial resistance detected between *P. multocida* and *M. haemolytica,* monitoring of resistance of bovine respiratory contributors should be pathogen-specific.

1202 - Is antimicrobial resistance driving the dissemination of Pasteurella multocida clones in cattle feedlots?

Author: Johan Manuel Calderón Bernal, Carlos Serna, Ángel García Muñoz, Alberto Díez Guerrier , Lucas Domínguez, José Francisco Fernández-Garayzábal , Ana Isabel Vela, María Dolores Cid

Objectives

Follow-up studies on beef cattle feedlots have been found, by pulsed field gel electrophoresis, that a few close genetically related clones (pulsotypes) of *Pasteurella multocida* are associated with clinical cases of BRD while these clones are not widely present in *P. multocida* isolates from healthy animals at the feedlot entry. The aim of this study was to investigate the possible genomic differences between these *P. multocida* prevalent clones from BRD-affected animals (BRD-AA) during the fattening period and those isolated from apparently healthy animals (AHA) at the time of entry into the feedlots in the same batches of the feedlot.

Material and methods

A total of 14 *P. multocida* isolates from different batches representative of the pulsotypes most frequently detected in BRD-AA (*n*= 6) and AHA (*n*= 8) were selected for further *in silico* multi-locus sequence typing (MLST) after their whole genome sequencing (WGS). In addition, 53 bovine *P. multocida* genomes belonging to ST13, ST79 and ST80, from different countries, available in the National Center for Biotechnology Information (NCBI) database, were selected for global comparison with the genomes of these STs identified in this study. Analysis of the 67 genomes included the detection of 27 virulence associated (VAGs) and antimicrobial resistance genes (ARGs), mobile genetic elements, and phylogenetic analysis.

Results

The genotypes identified in the 14 *P. multocida* isolates were ST79, ST13 and ST80, results consistent with those reported as most prevalent worldwide in BRD. Global comparison after WG-SNP phylogenetic analysis of the 67 genomes analyzed in this study showed that nearly all bovine genomes with the same MLST genotype clustered together in separated but closely related clusters. No differences in the detection of VAGs were observed in the 67 genomes of the ST13, ST79, and ST80 strains with most (21 of 27) of VAGs being uniformly detected in strains of the three STs. Information on the clinical or non-clinical status of the animals was available only for the 14 *P. multocida* isolates sequenced in this study. No differences in the contents of VAGs between clinical isolates and isolates from AHA within the same ST were detected that could favor the colonization of the respiratory tract or host–pathogen interactions.

The main difference among isolates of the three STs was the detection of ARGs conferring resistance to aminoglycosides, chloramphenicol, macrolides, sulfonamides and tetracyclines almost exclusively (except in one ST13 isolate) in the genomes of ST79 strains. The presence of ARGs was associated with the presence of putative integrative and conjugative elements (ICEs)which were present in 86.7% of the isolates of this ST. Two Spanish strains harbored an ICE highly similar (99.2% identity with a 100% coverage) to the ICE Tn*7407* of a *P. multocida* strain isolated from a fatal BRD case in Germany. This ICE is genetically related but distinct, to *ICEPmu1*, the ICE element most commonly detected in ST79 *P. multocida* isolates of bovine origin in North America.

Conclusions

Genomic comparison of *P. multocida* isolates from BRD-AA and AHA did not detect differences in the content of VAGs that could explain the higher frequency of detection of particular clones in BRD cases. The most significant finding was the detection in bovine prevalent *P. multocida* ST79 genotype of ARGs that encode resistance to different antimicrobial agents frequently used for the treatment of BRD bacterial infections, as tetracyclines and macrolides, that may have contributed to its selection and further dissemination among herds favored by the movement and mixing of animals from different origins.

1212 - Overview of an Ongoing Zoetis Antimicrobial Susceptibility Surveillance Program for Major Veterinary Pathogens in the United States and Canada

Author: Lacie Gunnett , Michael Sweeney, Dipu Mohan Kumar, Bryce Lunt, David J. Asper, Abhijit Gurjar, Véronique Moulin

Objectives

To continuously monitor for changes in susceptibility in antimicrobials used in veterinary medicine against target pathogens from across the United States and Canada.

Material and methods

Over 85,000 bacterial strains isolated as pathogens from beef and dairy cattle, pigs, horses, cats, and dogs have been collected by Zoetis since 1998 from diagnostic laboratories across the United States and Canada as part of a surveillance program designed to monitor the ongoing level of susceptibility towards the major antimicrobials used for specific disease indications in each of these animal species. Typically, thirty-three animal species/pathogen combinations are tested against sixty-two bacterial pathogen/drug combinations submitted from up to thirty-six laboratories each year. In order to limit over-representation of a geographical area, isolate submission from laboratories is limited to a maximum number of strains of each pathogen per year. Additionally, the number of isolates from a particular herd, ranch or household is limited to reduce the risk of overrepresentation of clones or epidemiologically related strains and subsequent data bias. Each isolate is considered by the submitting lab as the etiological agent responsible for disease. Isolates are randomly chosen by the laboratory without regard to susceptibility or previous antibiotic use. Laboratories are requested to include information regarding previous antibiotic use, when available. Minimal inhibitory concentration values for all isolates are determined using a broth microdilution system, which conforms strictly to the standards of the Clinical and Laboratory Standards Institute (CLSI) for testing veterinary pathogens and that is held to rigorous quality control standards beyond those recommended by CLSI.

Results

This long-term Zoetis surveillance program provides valuable information about numerical changes in *in vitro* activity of the most commonly used antimicrobials in veterinary medicine and provides a valuable and robust collection of historical strains of clinical pathogens. These data are extensive in geography and time; and for many animal species/drug combinations, more than twenty years of data have been collected across the United States and Canada using a consistent methodology.

Conclusions

Responsible stewardship of veterinary anti-infectives involves activities that aim to understand the development of resistance, minimize any effects of antimicrobial use, and sustain the efficacy of current veterinary drugs. Zoetis is committed to monitoring antimicrobial susceptibility through this surveillance program and surveillance for antimicrobial resistance among veterinary pathogens is an important component of stewardship advocated by Zoetis.

1214 - Emergence of Bovine Coronavirus as the Predominant Viral Respiratory Pathogen Detected by PCR in Cattle from Portugal.

Author: Geert Vertenten, Alfredo Teixeira, Adelaide Pereira

Objectives

Bovine respiratory disease (BRD) poses significant morbidity, mortality, and economic challenges to the cattle industry. While bovine respiratory syncytial virus (BRSV) and bovine parainfluenza type 3 virus (PI3) have been established as contributing agents to BRD, the involvement of bovine coronavirus (BCoV) has become evident in recent years. This study aimed to assess the prevalence and distribution of BCoV, PIV-3, and BRSV in respiratory samples collected from cattle across various regions of Portugal from 2019 to 2022.

Material and methods

We evaluated all viral polymerase chain reaction (PCR) results from respiratory swabs submitted between January 1st 2019 and December 31st 2022 and analyzed at SEGALAB (Laboratório de Sanidade Animal e Segurança Alimentar, Ponte de Lima, Portugal) using the same method of PCR analysis (Thermo Fisher VetMax Ruminant Respiratory Screening Kit) throughout the the study period. Nasal swabs were primarily obtained from cattle with clinical signs of bovine respiratory disease; however, individual animal data were not available.

Results

The prevalence of BCoV, PIV-3, and BRSV was determined in 415 respiratory samples submitted during the study period, with 117, 30, 11, and 35 samples from 2019, 2020, 2021, and 2022, respectively. In 2019, BRSV was the most frequently detected pathogen (37%), followed by BCoV (28%) and PIV-3 (13%). However, the order shifted in subsequent years, with BCoV becoming the most frequently detected pathogen, followed by BRSV and PIV-3. In 2020, the detection rates for BCoV, BRSV, and PIV-3 were 34%, 28%, and 13% respectively. In 2021, they were 29%, 24%, and 9%, and in 2022, they were 30%, 26%, and 9%. These data indicate a recent upsurge in BCoV as the most commonly detected viral respiratory pathogen in cattle, based on PCR analysis of respiratory swabs in Portugal.

Conclusions

Our findings confirm the presence of BCoV, PIV-3, and BRSV in cattle populations in Portugal from 2019 to 2022. Notably, BCoV was more frequently identified through PCR than other respiratory viral pathogens from 2020 to 2022. These results underscore the importance of sustained surveillance and control measures to mitigate the impact of these viral pathogens on the cattle industry in Portugal.

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1215 - A hard catch: Isolation of local pathogenic Leptospira from Austrian cattle

Author: Cynthia Sohm , Denise Willixhofer, Eva Fasching, Karoline Waldner, Nicole Deitzer, Janina Steiner, Julia Jöbstl, Corina Schleicher, Marcel Schwarz, Reinhard Fuchs, Pascale Bourhy, Annemarie Käsbohrer, Thomas Wittek, Clair Firth, Romana Steinparzer, Amélie Desvars-Larrive

Objectives

Leptospirosis is a neglected, globally spread zoonotic disease caused by pathogenic bacteria of the genus *Leptospira*.

In cattle, abortion and fertility disorders are commonly associated with leptospirosis and the disease can induce significant economic losses at farm level. People working in close contact with livestock, are at higher risk of infection.

Diagnosis of infection by *Leptospira* spp. is usually conducted via a serological test, the gold standard being the microscopic agglutination test (MAT). For this test a panel of live *Leptospira* strains, representing the main serogroups, are used. Both the World Organisation for Animal Health and the World Health Organization recommend the inclusion of locally circulating *Leptospira* strains in the panel to improve the test performance. However, no isolates from Austria have been available for inclusion in the MAT panel, used for the serological diagnosis of leptospirosis in both humans and animals, and the test has relied on non-native isolates.

The goal of this study was to isolate and genotype circulating *Leptospira* strains from cattle in Austria, to enhance the performances of the serological diagnostic for humans and animals. The findings of this study will also further our understanding of the local epidemiology of this zoonotic disease.

Material and methods

To maximise our chances of isolating *Leptospira* spp., we adopted a targeted sampling approach, focusing on animals considered at higher risk of carrying the bacteria, i.e. i) animals presenting with clinical signs compatible with leptospirosis and ii) animals originating from farms that were considered at high risk of leptospirosis. The inclusion criteria (i.e. individual and farm characteristics) were established based on a systematic literature review on bovine leptospirosis in Europe and a retrospective analysis of national bovine leptospirosis serological data. Additionally, the sampling strategy was complemented by a semi-targeted sampling approach.

Between November 2021 and September 2022, we collected urine samples from symptomatic cattle on farms as well as kidney tissue and, if logistically possible, urine samples, from slaughtered animals. All samples were inoculated into culture media and observed for leptospiral growth for at least 12 weeks. The samples were tested by PCR targeting the *lipL32* gene for the presence of pathogenic *Leptospira.* Cultures from PCR-positive samples were prioritised and monitored for up to 30 weeks before being discarded. The isolates where further genotyped by core genome multilocus sequence typing (cgMLST). Moreover, sequencing of the *lfb1* gene from PCR-positive samples was performed to determine *Leptospira* species.

Results

The systematic literature review and the statistical analysis identified symptomatic cattle as animals with a recent abortion, fertility disorders, icterus, photosensitisation,

haematuria, and/or a decrease in milk production. We included an animal if at least one symptom was present. Similarly, identified criteria for defining high-risk farms included: cattle replacements off-farm, access to pasture, large herd size, and history of abortions. We included animals from a farm when at least two of these criteria were present.
Overall, we obtained 429 samples from 410 cattle. Six urine and three kidney samples tested positive for pathogenic *Leptospira* spp. via *lipL32* PCR. Sequencing of the *lfb1* gene was successful in five urine samples and all of them were identified as *Leptospira borgpetersenii*.
We obtained three *Leptospira* isolates from the three PCR-positive kidney samples, representing a success rate of 33% in isolating *Leptospira* from PCR-positive samples. The leptospires exhibited slow growth, showing positive culture after 7-23 weeks. All isolates were identified as *Leptospira borgpetersenii* serogroup Sejroe serovar Hardjobovis belonging to the cgMLST cluster 40.

Conclusions

This study describes the first isolation and subsequent genotyping of zoonotic bacteria of the genus *Leptospira* in Austria. By isolating an autochthonous strain and making it available for the MAT panel, this study marks the initial step towards enhancing the performances of the serological diagnostics for both humans and animals within the country. Leptospirosis is commonly considered as a minor disease in both human and cattle Austria. Therefore, the isolation of *L. borgpetersenii* serovar Hardjobovis from cattle is a major finding, revealing that cattle may act as carriers of *Leptospira* in Austria and serve as a possible source of infection for humans, other animals, and the environment. Findings of this research suggest that, in case of abortion events in Austrian cattle, leptospirosis should be considered as a differential diagnosis.

1220 - Defining bovine leukemia virus-infected cows with high proviral load through lymphocyte count and quantitative real-time PCRs

Author: Junko Kohara , Shingo Asakura, Satoshi Nakada

Objectives
Bovine leukemia virus (BLV) is the causative agent of malignant lymphoma in cattle. BLV-infected cattle with persistent lymphocytosis have a high proviral load (H-PVL) and become infectious sources for BLV-free cattle on farms. This study aimed to determine cut-off value of BLV proviral DNA copy number to define the BLV-infected cattle with H-PVL using lymphocyte count and BLV PVL quantification.
Material and methods
Whole-blood samples were collected from 104 naturally BLV-infected Holstein-Friesian cows. Leukocyte count was measured using an automated blood cell analyzer. Genomic DNA was extracted from EDTA-treated whole-blood samples using QIAamp DNA Mini kit (QIAGEN, Tokyo, Japan). BLV PVL was measured using two different commercial quantitative real-time PCR (qPCR) kits, CoCoMo-LTR (Riken Genesis, Tokyo, Japan), targeting the BLV long terminal repeat (LTR) region, and RC201-pol (Takara Bio, Shiga, Japan), targeting the BLV pol region. Correlation between the lymphocyte count and the BLV PVLs were assessed by the Speaman's correlation coefficients. The cut-off values to detect BLV-infected cows with high H-PVL for both qPCR methods were determined using receiver operating characteristic curve analysis and employing the European Community (EC)'s key for lymphocytic status, which is based on the absolute lymphocyte count and age of a cow.
Results
Lymphocyte count and BLV PVLs measured by each qPCR were strongly correlated (CoCoMo-LTR; $r = 0.85$, $p < 0.0001$, RC201-pol; $r = 0.86$, $p < 0.0001$). The cut-off values of PVLs to define BLV-infected cows with H-PVL were calculated as 36,567 copies/100,000 cells for CoCoMo-LTR (area under the curve (AUC) = 0.95) and 30,868 copies/100,000 cells for RC201-pol (AUC = 0.956). The pPCR results of CoCoMo-LTR and RC201-pol showed a very strong correlation ($r = 0.98$, $p < 0.0001$). Out of the 104 BLV-infected cows, proportion of H-PVL cows defined by CoCoMo-LTR and RC201-pol qPCRs were 32.7% and 35.6%, respectively. Almost 100% of BLV-infected cows with lymphocytosis were classified as H-PVL cows by both CoCoMo-LTR and RC201-pol qPCRs.
Conclusions
The cut-off value of BLV H-PVL determined in this study could be used for defining high risk cows for source of BLV transmission and help to improve BLV control in dairy farms.

1221 - Lysosome-associated membrane protein 1 (LAMP1) enhances the antigenicity of DNA vaccine containing bovine viral diarrhea virus-1 (BVDV-1) E2 glycoprotein

Author: Yusuke Sakai, Shinji Yamada, Maho Inoue, Toshinori Shiga, Kei Kasai, Kenji Murakami

Objectives

Bovine viral diarrhea virus (BVDV) causes bovine viral diarrhea (BVD) and its infection causes respiratory symptoms, reduced milk yield, and abortion. Furthermore, calves of pregnant cows infected with BVDV are born as persistently infected (PI) cattle due to immune tolerance. PI cattle continue to shed the virus throughout their lives and are a major factor in the spread of infection on farms. BVDV causes significant economic losses to the global livestock industry. Culling of PI cattle and vaccination are important for controlling BVDV. The BVDV E2 protein is known as a protective antigen and is being investigated as a primary target for DNA vaccines against BVDV. DNA vaccines have various advantages, i.e., stability, easier to manufacture, and safer during handling. However, the immunogenicity of DNA vaccines is weak compared to existing live/inactivated vaccines and needs to be enhanced with adjuvant molecules or drug delivery systems. In this study, we focused on mouse lysosome-associated membrane protein 1 (mLAMP1) as a molecular adjuvant and developed the DNA vaccine encoding mLAMP1 and BVDV E2 chimeric gene (pVax-mLAMP1-E2).

Material and methods

The mLAMP1 molecule is a membrane glycoprotein composed of two functional domains and a hinge region. DNA plasmids were constructed in which the BVDV E2 gene was inserted within the hinge region (H) or membrane proximal domain (D) of mLAMP1 gene. The mLAMP1-BVDV E2 fusion protein is expressed by inserting the BVDV E2 gene into the mLAMP1 full-length gene. When these plasmids were transfected into cultured cells, higher expression of E2 antigen was confirmed in pVax-mLAMP1-E2 (H) with the E2 gene inserted within the hinge region. Mice and cattle were immunized intradermally and/or intramuscularly with pVax-mLAMP1-E2 (H), and viral neutralization test and E2 antigen-specific IFN-γ production assay were performed.

Results

After immunization with the DNA vaccine twice, neutralizing antibody titers against BVDV in mouse serum and antigen-specific IFN-γ secretion in splenocytes were evaluated. Mice immunized intradermally with pVax-mLAMP1-E2 (H) induced sufficient neutralizing antibodies and their splenocytes showed E2 antigen-specific IFN-γ production. In cattle, serum neutralizing antibody titer injected intradermally or intramuscularly with pVax-mLAMP1-E2 (H) tended to be higher than with pVax-E2. In addition, E2 antigen-specific IFN-γ secretion was confirmed in PBMCs from cattle immunized intradermally with pVax-mLAMP1-E2 (H).

Conclusions

These results suggest that LAMP1 fusion antigens may effectively enhance humoral and cellular immunity, especially when the antigen is inserted in the hinge region. The results of this study are the first to demonstrate the usefulness of LAMP1 molecules as

a molecular adjuvant for DNA vaccines for cattle. LAMP1-E2 fusion DNA vaccines may be useful candidates as BVDV vaccines in cattle.

1225 - Study on clinical scoring, lung ultrasonography and post mortal lung inspection for diagnosis of bovine respiratory disease in veal calves

Author: Thomas Wittek , Walter Pienhopf-Petz, Julia Hoffelner

Objectives
Respiratory disease in calves is often accompanied by an extensive usage of antimicrobial drugs. As part of the strategy to reduce antimicrobial resistance by “prudent use of antibiotics”, veterinarians and farmers need to ensure that individual therapy decisions are based on a “prior risk assessment” of the severity and prognosis of the respiratory disease.
The objectives of this study were first to evaluate the extent and severity of lung diseases clinically and by ultrasound in calves in an Austrian veal farm at two time periods: 1. on arrival at the beginning of the fattening period in calves of approx. 6–8 weeks of age and 2. at the end of the fattening period approx. 80–120 days later. Second, these clinical and ultrasonographic data were compared to a visual assessment of lung lesions after slaughter, allowing the evaluation of the diagnostic and prognostic value of these examinations.
Material and methods
The study period had a duration of one year. In total, 600 veal calves (150 per season) were examined at the beginning and the end of the fattening period. A physical examination was performed using a scoring system and a thorax ultrasonography was performed. After slaughter the lungs were examined. The associations between examination methods were calculated with Spearman rank correlation coefficients (rsp), further the diagnostic values were calculated using sensitivity, specificity, positive predictive value and ROC curve analysis
Results
Overall, the area under the curve (AUC) for ultrasonographic scores was 0.90 (rsp = 0.78) with a sensitivity (Se) of 0.86. The specificity (Sp) was 0.78, and the positive predictive value (PPV) was 0.74. The AUC for the physical examination was 0.76 (rsp = 0.55) with a Se of 0.64, an Sp of 0.81, and a PPV of 0.69. For the combination of ultrasonography and physical examination, an AUC curve of 0.85 (rsp = 0.69) was calculated. A Se of 0.65 and a Sp of 0.88 with a PPV of 0.73 was calculated.
Conclusions
This study concluded that both physical and ultrasonographic examination scoring are reliable examination methods for the detection of lung diseases in veal calves. The study highlights the importance of an early diagnosis of respiratory disease and shows that ultrasonography of the thorax is useful for early detection of lung tissue changes and should assist in treatment decisions reducing the use of antibiotics.

1255 - Conidiobolomycosis in sheep (Ovis aries) associated with Conidiobolus lamprauges in Colima, Mexico

Author: Jorge Luis García Valle, Johnatan Ruíz-Ramírez, Rafael Macedo Barragán, Jorge Pineda-Lucatero, Victalina Arredondo-Ruiz, Luis Jorge García-Márquez

Objectives

The objective of this study was to describe clinicopathological aspects of conidiobolomycosis in sheep (*Ovis aries*) caused by *Conidiobolus lamprauges* in Colima, Mexico.

Material and methods

Ten Pelibuey sheep (eight females and two males) with ages ranged between two and three years presenting bilateral muco-hemorrhagic rhinitis were referred to the Laboratory of Pathology of the University of Colima to carry out the corresponding necropsies. Sheep presented a respiratory semiology with more than five months of evolution with clinical signs including poor body condition, anorexia, apathy, depression, dyspnea, tachypnea, nasal stridor, cyanotic mucous membranes, mucopurulent and bilateral hemorrhagic nasal discharge with nasal deformation and unilateral exophthalmia. Mid-sagittal sections of the head were made to observe and describe the lesions affecting the ethmoidal region, turbinates, paranasal sinuses and soft and hard palate. Samples were fixed in 10% buffered formalin to process them with the routine histological methods. Sections of 5 µm were stained with Hematoxylin-Eosin (H-E), Periodic acid-Schiff (PAS), Grocott-Gomori´s methanamine silver (GMS) and Ziehl Neelsen (ZN). The diameters of the hyphae were measured and for the immunohistochemistry test (IHC), primary antibodies against *Pythium insidiosum* and *Conidiobolus lamprauges* were used.

Results

Lesions were located in the caudal, dorsal and ventral regions of the head affecting the ethmoid, turbinates, epithelium, bone and cartilaginous tissue of the rhinopharyngeal region which were occupied by neoformation tissue with caseous and liquefactive necrosis, which was granular, friable and irregular in appearance, yellow-brown (fibrin) to dark red (necrosis) in color, with blood clots interspersed with mucopurulent exudate that ranged from 3 to 16 cm along the rostral axis and from 1 to 10 cm dorsoventrally. The bone tissue was replaced and softened (osteonecrosis). Nasal mucosa was replaced by extensive necrotic areas with numerous cellular and karyorectal debris, interspersed with fibrin, lymphocytes, plasma cells, macrophages, multinucleated giant cells (granulomas) and neutrophils, with multifocally coalescent areas of pyogranulomas. Intralesionally there were numerous short, irregular and filamentous hyphae poorly stained with H-E while with (GMS) hyphae were seen wide (3 to 25-µm) and irregular, with parallel walls and lacking septation (aseptate) and branches (Splendore-Hoeppli material). Some blood vessels presented vasculitis and thrombosis. The final diagnosis was severe dorso-ventral caudal pyogranulomatous rhinitis. The immunohistochemistry test revealed positivity for *Conidiobolus lamprauges*, the brown, intralesional, sparsely septate hyphae within the coalescent necrotic areas and negative for *Pythium insidiosum.* No lesions were observed in other tissues.

Conclusions

It is concluded that conidiobolomycosis caused by *Conidiobolus lamprauges* causes significant damage in the sheep production chain and makes clear the importance of knowing the clinical, pathological and epidemiological scenarios of rhinitis in sheep to implement prophylactic control, prevention and biosafety measures, particularly in the tropical regions of Mexico.

1257 - Ovine gammaherpesvirus 2 can serve as an agent of bovine respiratory disease

Author: Selwyn Headley Arlington, Juliana Torres Tomazi Fritzen, Ana Laura Paulino Leite Gomes, Maria Constanza Rodriguez, Flávia Helena Pereira Silva, Maria Izabel Pedra Sogari, Ana Aparecida Correa Xavier, Allana de Andrade Mathias, Priscila Fajardo Valente Pereira, Thais Helena Constantino Patelli, Giovana Wingeter Di Santis, Julio Augusto Naylor Lisboa, Amauri Alcindo Alfieri

Objectives

The *Macavirus*, ovine gammaherpesvirus 2 (OvGHV2), is the cause of sheep associated-malignant catarrhal fever (SA-MCF). All *Macavirus* known to cause MCF share the 15A epitope, are referred to as malignant catarrhal fever virus (MCFV) and are conserved within the DNA polymerase gene (Li et al., 2003). Ruminants with SA-MCF may develop several clinical syndromes including the more frequent head-and-eye form, as well as alimentary, neurological, and cutaneous manifestations. Bovine respiratory disease (BRD) is a multietiological and multifactorial syndrome that is associated with several bacterial (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni,* and *Mycoplasma bovis*) and viral [bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine alphaherpesvirus 1 (BoAHV1), bovine parainfluenza virus-3 (BPIV-3), and bovine coronavirus (BoCV)] disease agents, coupled with management and environmental factors. Our group postulated that OvGHV2 should be considered a potential pathogen of BRD since initial viral replication occurs in the pulmonary epithelial cells of infected animals (Headley et al., 2020). Additionally, several studies have confirmed the participation of a MCFV and/or OvGHV2 in the development of pulmonary disease in cattle (Oliveira et al., 2021; Headley et al., 2023). This report summarizes studies that investigated the possible role of OvGHV2 in the development of BRD.

Material and methods

Biological samples (nasal swabs, n=11; pulmonary tissue, n=22) were obtained from cattle reared in Southern Brazil with alimentary (n=21), respiratory (n=7), and neurological (n=3) clinical disease syndromes and from asymptomatic cattle (n=2). All lung samples were processed for routine histopathology to determine the pattern of pulmonary disease. Pulmonary fragments were used in immunohistochemical (IHC) assays designed to detect intralesional tissues antigens of MCFV using the 15A monoclonal antibody (15A-MAb). All biological samples were used in molecular assays to amplify specific genes of *M. haemolytica*, *P. multocida*, *H. somni*, *M. bovis*, BVDV, BRSV, BoAHV1, BPIV-3, BoCV, OvGHV2, and bovine gammaherpesvirus 6 (BoGHV6), another *Macavirus*. Positive and negative controls were included in all assays.

Results

From the six animals with clinical manifestations of BRD, most (83.3%; 5/6) were infected by OvGHV2 and two of these by BoGHV6; one of these cows was simultaneous infected by both *Macavirus*. Two patterns of pulmonary disease were identified: interstitial pneumonia, IPN (n=21) and suppurative bronchopneumonia (n=5), with the latter being identified in all animals with IPN. There was positive intracytoplasmic immunoreactivity to the 15A-MAb IHC assay within epithelial cells of the lungs of all

cattle with IPN, confirming that these were infected by a MCFV. Only nucleic acids of OvGHV2 (n=23), BoGHV6 (n=11), *H. somni* (n=4), BCoV (n=3), and BoAHV1 (n=1) were amplified from the biological samples evaluated. When the relationship between IPN and infection was evaluated, most cattle were infected by OvGHV2 (n=11) and BoGHV6 (n=9). Additionally, only three of the agents detected in the pulmonary tissues were identified in the nasal swabs (OvGHV2 DNA, n=12; BoGHV6, n=2; and BCoV, n=1). Furthermore, OvGHV2 (n=6) or BoGHV6 (n=4) DNA or DNA from both of these agents simultaneously (n=6) were detected in the lungs of most animals with IPN and with positive immunoreactivity to the 15A IHC assay, except for five animals. Moreover, during this study 22 singular infections were identified; most of these being associated with OvGHV2 (n=15), followed by BoGHV6 (n=4). Dual infections were due primarily to OvGHV2 and BoGHV6 (n=5); one animal was simultaneously infected by BoGHV6 and BCoV. In addition, one triple (OvGHV2 + BoGHV6 + *H. somni*) and one quintuple (OvGHV2 + BoGHV6 + BoAHV1 + BCoV + *H. somni*) infection was identified.

Conclusions

This study demonstrated that most cattle with clinical manifestations of BRD and histopathological evidence of were infected by OvGHV2, resulting predominantly in singular and concomitant infections with other agents of BRD. Consequently, these findings indicate that OvGHV2 acting alone or in conjunction with other agents of cattle can produce clinical manifestations and histopathological lesions of BRD, suggesting that this agent should be considered as a potential inductor of respiratory disease in cattle. The identification of singular infections by BoGHV6 in animals with IPN may suggest that this virus is not a commensal agent of cattle. Additionally, the nonidentification of OvGHV2 and BoGHV6 DNA in five animals with IHC evidence of MCFV infection, may suggest the circulation of a previously undetected *Macavirus* in cattle herds from Brazil.

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1272 - Certification of dairy herds based on their paratuberculosis status in the Czech Republic

Author: Vera Fichtelova , Alena Kralova, Kamil Kovarcik

Objectives

Mycobacterium avium subs. *paratuberculosis* (MAP) is the causative agent of paratuberculosis (PTB), chronic granulomatous enteritis that eventually leads to death in infected animals. PTB is generally considered a health and economic concern within the dairy cattle industry. Although the exact prevalence among Czech dairy herds is unknown, it is presumed to be high due to the relatively frequent occurrence of herds with positive results in the bulk tank ELISA (Enzyme-Linked Immunosorbent Assay) milk tests.

To reduce prevalence, it is essential to prevent the transmission of infection within and between herds. Therefore, control programmes are implemented in herds. Farmers are the primary participants in PTB control, and knowledge of the PTB status in herds enables them to introduce appropriate control measures. It also reduces the risk of purchasing infected cows from farms with a high prevalence of infection.

The aim of this study is to present the certification programme for dairy herds based on their PTB status in the Czech Republic.

Material and methods

Three different diagnostic methods were selected according to their varying levels of sensitivity to detect MAP-infected herds: bulk tank milk (BTM) ELISA (>10% of Ab positive animals present in the herds), qPCR (quantitative polymerase chain reaction) of environmental samples (ES) (<3-4% of MAP shedding animals present in the herd), and individual serum/milk ELISA (identification of herds with ≤2% of Ab positive animals present in the herd).

Results

Four (D-A) statuses have been proposed to classify herds according to their risk of PTB infection: D-herds with a high risk of PTB, this status is assigned to herds with positive ELISA results from BTM or herds with no investigation to detect MAP infection (unknown herd status); C-herds with a medium risk of PTB, this condition is determined by the absence of Ab in BTM along with the detection of MAP in ES; B-herds with a controlled risk of PTB, this status clusters herds with no MAP detection in ES; A-herds with a low risk of PTB, in these herds, the prevalence of Ab-positive animals is under 2%.

Controlled herds are continuously tested. Five consecutive, negative BTM ELISA or ES qPCR results within 3-7 months are required to achieve status C or B, and herds must be repeatedly tested with an appropriate diagnostic method at 10-14 months intervals to maintain the achieved status. To achieve A status, three consecutive negative serum ELISA results within 10-14 months or five consecutive negative milk ELISA results within 3-7 months (2 per year) are required for all cows aged 24 months and older. After reaching A status, the serum or milk of all cows aged 30 months and older is repeatedly tested by ELISA at intervals of 10-14 months. Animals that test positive should be removed within 6 months of a positive test result or after a positive confirmatory test performed within 31 days from the initial ELISA test.

The PTB herd certification application was created and provided to farmers on the server of ČMSCH a.s., a Czech service organization for all livestock holders. This organization is

also responsible for milk and ES sampling for certification and acts as the PTB certification authority.

Participation in the certification is voluntary and farmers can choose the final status they want to achieve.

Conclusions

PTB certification serves farmers as a useful tool for assessing the risk of PTB presence in their herds. Knowledge of the PTB status of purchased cattle is particularly important to minimize the risk of introducing infection into the herd. The efforts of farmers to improve and also maintain the achieved PTB status further compel them to adopt control measures to reduce the transmission of infection to susceptible animals.

Introduction of herd certification based on their PTB status may increase farmers' motivation to control PTB in cattle.

1273 - The use of serum ELISA and qPCR for analysing pooled faecal samples to detect MAP-infected cows in beef herds

Author: Vera Fichtelova, Alena Kralova , Vladimir Babak, Kamil Kovarcik

Objectives

Mycobycterium avium subsp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis in cattle (Johne's disease). Infected animals go through four disease stages: latent, subclinical, clinical, and advanced, with progressive MAP shedding and Ab production in the last two stages. Identifying and removing MAP-shedding animals is a cornerstone in reducing the transmission of infection among susceptible animals. Shed MAP can be detected in faeces using polymerase chain reaction (PCR), quantitative PCR (qPCR), or bacterial cultivation. The infection is diagnosed indirectly through the detection of specific antibodies using the Enzyme-Linked Immunosorbent Assay (ELISA) test. Shedding of a small amount of MAP precedes Ab production. PCR testing of individual or pooled faecal samples (PFS) and the serum ELISA test are both routinely used to detect infected animals. The objective of the present study was to evaluate qPCR of faecal samples and serum ELISA to detect infected animals in beef herds.

Material and methods

763 cows, 2 years old and older, originating from 6 beef herds (57 to 303 animals per herd, average 127 cows) were included in the study. Blood and faecal samples were collected from all animals.

Serum from clotted blood was tested for the presence of anti-MAP antibodies by ELISA (Test Line, CZ). Equal amounts of individual faecal samples were thoroughly mixed to prepare pooled faecal samples, with a maximum of five samples. DNA of PFS, individual faecal samples from qPCR positive pools, and faeces from ELISA-positive cows was extracted and tested in qPCR, amplifying the specific ISMav2 region of the MAP genome. Faecal samples from cows that tested positive by qPCR and/or ELISA were cultured on solid Herrold's egg yolk medium with mycobactin following the OIE (World Organisation for Animal Health) manual 2018.

The statistical significance of the difference between two proportions was assessed by a two-sample test for proportions.

Results

Considering the method used to detect infected cows, 34 and 32 positive animals were identified by ELISA and qPCR of the pooled faecal samples, respectively. 27 cows tested positive in both tests. Out of these double positive cows, 16 were detected using qPCR of PFS and all 27 were detected by ELISA. A positive result was identified in only one test, either in qPCR or ELISA, for sixteen and seven animals, respectively.

Overall, fifty cows with positive qPCR and/or ELISA test results were detected, of which 34 (68%) were ELISA positive and 43 (86%) were qPCR positive. From these 43 qPCR positive samples, 32 were detected due to the investigation of PFS, with subsequent testing of individual faeces included in positive pools. The remaining 11 qPCR positive samples were detected through qPCR testing of faeces from cows previously diagnosed as ELISA positive.

Bacterial culture detected MAP in 26 faecal samples, with 21 (80.8%) originating from cows with positive results in both qPCR and ELISA tests. The remaining 5 culture-positive

faecal samples belonged to cows with only qPCR-positive results, and no MAP was detected in the faeces of cows with only ELISA-positive results.
A statistically significant difference was revealed when comparing qPCR of PFS and ELISA to detect double-positive animals.

Conclusions

Fifty animals with positive results in at least one diagnostic test were detected among 763 beef cows. To minimize the probability of misclassification of infected animals as not infected, and vice versa, repeated testing and/or the use of two or more independent tests are recommendable. 27 cows had positive results in both tests, all of which could be detected by ELISA, while 11 were not been detected by the investigation of PFS using qPCR. These double-positive animals are considered to be truly infected and should be removed from the herd. This decision is further supported by results of bacterial cultivation, where viable MAP was detected in almost all faeces of these cows. Based on our results, it is recommended to detect infected beef cows using the ELISA test, followed by qPCR testing of faeces to improve specificity of testing (confirmatory assay).

1275 - Assessment of the prevalence of Streptococcus uberis in dairy farms in México

Author: Gabriel Ruiz, Rui Cepeda , Ariel Hernandez , Dolors Giralt

Objectives
Streptococcus uberis has been reported as one of the most prevalent causative agents of mastitis in many countries. Given its importance in udder health and dairy farming, the knowledge of its epidemiology is crucial to develop proper control strategies.
The objective of this study was to describe the incidence of *S. uberis* mastitis in dairy farms in Mexico.
Material and methods
The data concerning *S. uberis* milk tank PCR analysis was gathered from 30 farm located in Bajío, Mexico. This data concerned the period between February 2018 and February 2023. To compare positivity regarding seasonality a logistic regression was performed using R software v4.3.
Results
Throughout the entire study period a total 1257 bulk tank milk samples were collected and 73.27% were positive to *S. uberis*. When these results were stratified by year, it was observed that during 2018 and 2019 the percentage of positive samples was maintained between 74.45 % and 73.72 %; in 2020 this value dropped to 62.33 % to then after steadily increase up to 81.14 % in 2023. The data was then further stratified, based in the seasonality: wet or dry. Results showed that the percentage of positive cases were higher in the wet season compared to dry season, this indicating an association between this factor and *S. uberis* $p<0.001$).
Conclusions
The results of this study showed that *S. uberis* might be one of the most prevalent pathogens causing mastitis also in Mexican dairy farms. Several factors may be influencing the prevalence of this pathogen in mastitis cases; based on the results of this study, seasonality is significatively associated with *S. uberis* prevalence. Despite it is difficult to predict the future outcome of *S. uberis* prevalence, this study indicates an increasing trend of this pathogen.

1317 - Biosecurity status of dairy farms included in national targeted advisory services on animal health (TASAH) programmes.

Author: Siobhan O'Donovan, John Mee F, Alison Burrell, Conor McAloon, Luke O'Grady, Tim Geraghty

Objectives

Farmers engaged in a targeted advisory services on animal health (TASAH) might be expected to have better than average farm biosecurity status. In Ireland, the national animal health organisation, Animal Health Ireland (AHI) provides such services to livestock farmers. Hence, the objective of this study was to assess the biosecurity status of Irish dairy herds enrolled in two AHI animal health advisory programmes: Parasite TASAH group (Para) and the Irish Johne's Control Programme group (IJCP).

Material and methods

An online biosecurity survey with 75 questions was sent via email to all members of the two groups (1,637 Para; 1,635 IJCP) in April 2023. The questionnaire was developed as described in O'Donovan *et al.,* (2024)

Results

In total, 150 dairy farmers responded, 51 Para and 99 IJCP, (3.1% and 6% response rates). On bioexclusion practices: 43% (16% Para; 27% IJCP) of farmers declared their herd "closed". Just 13% of respondents bought animals from either a mart (6%; 4% Para; 2% IJCP) or auction/show and sale (7%; 2% Para; 5% IJCP). Half of both the IJCP and the Para group respondents did not enquire about the Johne's disease (JD)/paratuberculosis status of animals purchased. The majority [82% (26% Para; 56% IJCP)] of dead stock collectors used no disinfection measures upon visiting farms, followed by 42% (10% Para; 32% IJCP) of hoof trimmers, 26% (8% Para; 18% IJCP) of AI technicians, 20% (5% Para; 15% IJCP) of ultrasound scanner operators and 14% (3% Para; 11% IJCP) of veterinarians. On biocontainment practices: 19% (6% Para; 13% IJCP) of farmers had no dedicated isolation pen for sick animals while a further 36% (15% Para; 21% IJCP) said they had an isolation pen however, all animals had shared airspace.

Conclusions

Encouragingly most high-risk visitors practised on-arrival disinfection. Of concern was the prevalence of open herds, failure to enquire about purchase animal JD status and lack/quality of isolation facilities. These findings indicate the need for further engagement with dairy farmers in TASAH programmes about their biosecurity status and practices.

Reference

O'Donovan, S., O'Grady, L., McAloon, C., Geraghty, T. and Mee, J.F. (2024) Development of a novel combined biosecurity risk assessment scoring tool and cattle movement audit. *Proceedings of the 32nd World Buiatrics Congress*, Cancun, Mexico.

1320 - Mycoplasma bovis apparent prevalence and association with Bovine Respiratory Syncytial Virus in French dairy and suckler cow/calf productions

Author: Stéfanie Bernheim -, Camille Levesque Ninio, Yves Millemann

Objectives

Bovine Respiratory Disease (BRD) is a major cause of economic loss, impacting animal welfare and leading to intensive antimicrobial use in cattle operations. BRD is a multifactorial infectious disease caused by a complex interaction between different viral and bacterial pathogens. This study aimed to investigate the frequency of detection of two main etiological agents involved in BRD and their possible association with BRD outbreaks. *Mycoplasma bovis* is now considered as a primary respiratory pathogen that can have important productivity impact. Necropsic examination of the lungs is key in providing etiological hypothesis that can be confirmed by PCR analysis. The objective of this study was to determine the detection frequencies of *Mycoplasma bovis*, using RT-PCR on individual lung samples, regarding the type of production and possible association with BRSV infection.

Materials and methods

1033 bovines were included in a field study to monitor the etiology of respiratory mortality in France between January 2020 and November 2023. Even if BRSV intranasal vaccination interference with PCR has not been demonstrated, vaccinated animals had been excluded. Dead animals were necropsied after clinical acute or chronic respiratory signs. In the framework of a diagnostics support service provided by Zoetis (Rispaudit) Real time PCR Vet-MAX™ Ruminant Respiratory Screening Kit (Thermofisher Scientific) was used by the Labocea French laboratory to detect *Mycoplasma bovis* and BRSV. Validated samples were either frozen or refrigerated lung pieces, or fresh lung transferred on FTA cards. These Flinders Technology Associates (FTA®) elute cards have been treated to inactivate the virus or bacteria transferred on them by contact. They can be stored at room temperature. This means that the material taken can be transported safely and easily to the laboratory in order to detect pathogens genetic material with for instance PCR studied samples were collected from dairy (23.3%), cow-calf (59.6%), feedlots (12.1%) and veal calves (5%) production systems and were all analyzed individually. The breakdown for each category between lung samples and FTA cards is as follows: 60/40% for dairy, 57/43% for cow-calf, 65/35 for veal calves and 40/60 for feedlots. With a significant difference for feedlots vs the other productions ($p<0.001$) as FTA cards have been more used in this segment.

Results

The *Mycoplasma bovis* genome detection frequency on lungs was 20 % in the dairy and cow-calf groups with no significant difference (p=0,8), 73% in the feedlot group and 57% in the veal calves one. Except between dairy and cow-calf (p=0,8), differences are all significant (p<0,0001). Among the *M. bovis* positive samples the proportion of samples also positive for BRSV genome was evaluated. 32% of the Dairy *M. bovis* positive lung samples were also positive for BRSV. This proportion was 39.3% for cow-calf, 49.4% for feedlots and 24% for veal calves, with a significant difference between veal calves and feedlots (P=0,01).

Conclusions

These results confirm that two major respiratory pathogens that, whatever the bovine production segment, impair the global health, welfare and productivity of youngstock, namely Bovine Respiratory Syncytial Virus and *Mycoplasma bovis* are frequently detected in those production systems in France. Even if apparent prevalences depend on the production type, each of them is impacted with *a minima* one infected herd on 5 to a maximum of 3 feedlots on 4. Detection on lung tissue eliminates the question of possible healthy carriage in the upper respiratory tract. These detection levels should lead to the implementation of combined and appropriate vaccine prevention strategies.

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1327 - Risk factors associated with salmonellosis and Mycoplasma mastitis in dairy farms in Hokkaido, Japan

Author: Shingo Asakura , Shigeo Fukuda, Yuki Hirano, Yoshie Sakurai, Junko Kohara

Objectives
The risk of within farm infection and negative effects of bovine infectious diseases have become huge as the number of dairy cattle per farm has been increasing in Japan. This study aimed to explore factors associated with salmonellosis and Mycoplasma mastitis in dairy farms in Hokkaido, the center of dairy farming in Japan.
Material and methods
A case-control study was conducted in the Tokachi and Nemuro Regions of Hokkaido from 2018 to 2020. The study included 14 farms with cases of salmonellosis and 27 without, and with 15 farms with Mycoplasma mastitis cases and 26 without, within the previous three years at the time of survey. Data were collected through questionnaire survey to farm owner or worker. Logistic regression analysis was conducted using the data as explanatory variables and disease occurrences as response variables, adjusting for the number of cattle as a confounder. Missing numerical data were imputed using multiple imputation.
Results
Factors considered biologically plausible with low p-values included the presence of bunker silos for salmonellosis (Adjusted Odds Ratio [AOR] = 5.38, 95% CI: 0.99-28.97, p=0.05), with slurry treatment of manure (AOR = 0.11, 95% CI: 0.01-1.00, p=0.05) and pre-composting manure export (AOR = 3.87, 95% CI: 0.87-17.14, p=0.07). For Mycoplasma mastitis, poor bovine body hygiene was identified as a significant risk factor (AOR = 3.23, 95% CI: 1.17-8.93, p=0.02).
Conclusions
The study suggests that for salmonellosis, the presence of bunker silos could increase the risk of pathogen spread by attracting wild birds and animals. In addition, improper manure processing also could contribute to the risk. In Mycoplasma mastitis, poor bovine body hygiene was considered to be associated with udder hygiene which is critical for the disease. The study has limitations in sample size and detection power, necessitating cautious interpretation of the results and underscoring the need for further in-depth research.

1328 - The effect of a Mycoplasma bovis modified live vaccine on Average Daily Gain in a French feedlot

Author: Stéfanie Bernheim -, Alfredo Zanini, Leentje Dreesen, Ely Benere, Vanessa Dablin, Barbara Descamps, Suman Mahan, Abhijit Gurjar, David Asper, Véronique Moulin

Objectives

Mycoplasma bovis (M. bovis) is considered an important pathogen of cattle, causing disease with detrimental impacts on animal welfare and the economics of livestock production[1]. With high incidence rates[2], *M.bovis* is one of the major pathogens found in feedlots. Disease associated with *M. bovis* in young calves presents as pneumonia, otitis media, arthritis, or any combination thereof, with an onset typically reported between 2 and 6 weeks of age. In addition, *M. bovis* is an important contributor to the complex of pathogens involved in the development of Bovine Respiratory Disease (BRD) in older cattle[3]. Clinically BRD due to *M. bovis* tends to become chronic and debilitating, leading to increased use of antimicrobials. Chronic cases usually grow poorly leading to low value animals at slaughter, which significantly impacts farm productivity. As a possible alternative to current methods of control of *M. bovis*, [Protivity®(Zoetis)] a *M. bovis* Modified Live Vaccine is available in France since 2022 and can be used for vaccination of healthy cattle from 1 week of age. It is proven to be effective against respiratory disease caused by *M. bovis*, with reduction of clinical signs and lung lesions. A retrospective study was conducted in a French feedlot to monitor the impact of vaccination with [Protivity®] on Average Daily Gain (ADG) expressed in kilograms per day (kg/d).

Material and methods

4078 limousin calves fattened for an average of 156 days were monitored from 2020 to 15th November 2023. On a weekly basis, 8 to 12 month old calves were bought from local markets, and comingled on the fattening site, where they were sorted to groups of 11, and quarantined for a period of 3 weeks. From November 2021, a BRD outbreak started, due to *M. bovis* which was identified by qPCR and bacteriology. With no satisfying therapeutic response to antimicrobials, it was decided to vaccinate new arrivals against *M.bovis*. From March 2022, *M. bovis* vaccination with 2 doses of [Protivity®] of 2 mL (SC), 21 days apart, was added each week to the new arrivals vaccination historical protocol [Rispoval3®(BRSv, Bpi3v, BVDv, 4mL IM, Zoetis), Hiprabovis somni LKT® (*Mannheimia haemolytica, Histophilus somni*, 2mL SC, Hipra)]. A total of 5 months were needed to fully immunize all 450 calves present on the farm. ADG was evaluated from the available data and consisted of individual body weights at arrival and departure, and days in feed

Results

2020 was considered as the reference year with 1089 fattened calves without any abnormal health incidence rate. In 2021, 1126 calves were fattened, 966 in 2022, and 897 in 2023 until 15th of November. The ADG for 2020 was 1.6kg/d. In 2021, ADG was also reported to be 1.6kg/d with two months of a BRD outbreak. In 2022, during the first year of introduction of vaccination with [Protivity®], ADG increased to 1.7kg/d and kept constant through 2023. This ADG difference of 100g was significant when comparing 2020/2021 to 2022 ($p<0.0001$) and 2020/2021 to 2023 ($p<0.0001$). To evaluate the

impact of vaccination on the chronic incurable case rate, the proportion of animals with ADG under 1.3kg/d was also calculated. In 2020, considered as a healthy year, and in 2021 the rate was respectively 20% and 21% with no significant difference ($p>0.05$). In 2022 the rate was 11.5% and in 2023 it was 13.9% with a significant difference when compared to 2020/2021 ($p<0.001$).

Conclusions

The growth of cattle in feedlots is influenced by various factors, primarily guided by the results of feed management, prevention protocols, and health treatment interventions. Over the 4 years study period only minor adjustments took place regarding feeding procedures and composition. The main additional element that has been identified was the introduction of *M.bovis* vaccination with [Protivity®], which significantly improved the feedlot disease management health situation, with a correlated positive impact on the average ADG, and therefore a significant gain on farm revenues

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1331 - Co-morbidity associated with infection by Anaplasma phagocytophilum (tick-borne fever) - Final

Author: Ciara McDermott, John Mee F, John Gilmore, Shane McGettrick, Rebecca Froehlich, A Flaherty, Colm O'Muireagain, C Harte, Seamus Fagan, Maresa Sheehan, James O'Shaughnessy, Tim Keady, Annetta Zintl

Objectives

Tick borne fever (TBF) is a tick-transmitted disease in ruminants caused by the Gram-negative bacterium *Anaplasma phagocytophilum.* Clinical signs include pyrexia, depression, immunosuppression and increased susceptibility to diseases such as Louping Ill and pasteurellosis[1]. This study aimed to investigate comorbidities that were typically associated with TBF in lambs from different age groups and locations throughout Ireland.

Material and methods

The study included lamb carcasses under one year of age submitted to three national veterinary laboratories (Athlone, Kilkenny, and Sligo Department of Agriculture, Food and the Marine Regional Veterinary Laboratories) between 1st January 2021 and 31st December 2022. Aborted foetuses were excluded. Spleen samples were tested for the presence of *A. phagocytophilum* using qPCR and grouped into positive and negative categories. Clinical histories and postmortem findings were retrieved from the Laboratory Information Management System (LIMS) and collated onto a Microsoft Excel Worksheet following an anonymisation process. Comorbidities of positive tests determined by veterinarians from post-mortem and laboratory findings were classed by pathogen, aetiology and organ system affected.

Results

Out of a total of 871 submissions, 54 were positive for TBF (6.2%). Pneumonia was diagnosed in 24% of positive cases, where *Mannheimia haemolytica* was the primary agent in 75% of these pneumonias. Pneumonia was diagnosed in 13.7% of negative cases, with *M. haemolytica* isolated in 43.6% of pneumonias. In a previous survey of ovine mortality in Ireland in 2016, pneumonia was diagnosed in 10.5% of lambs, with *M. haemolytica* isolated in 30.8% of pneumonias.[2]

Conclusions

These results indicate that infection with TBF may have a positive association with pneumonia and with infection by *Mannheimia haemolytica*. The author would like to acknowledge funding from the Irish Department of Agriculture, Food and the Marine.

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1339 - Phylogenetic inferences of viruses with respiratory tropism in ruminant production units of México City

Author: Jose Francisco Rivera Benitez, Jazmín De La Luz, Andrés Ducoing, Eduardo Cabrera, Georgina Hernández

Objectives
The most important viral agents in the respiratory complex in ruminants (ovine, caprine and bovine) are respiratory syncytial virus (RSV), parainfluenza virus type 3 (PI3V) and bovine viral diarrhea virus (BVDV), and been reported to have a worldwide distribution in these three species. The objective of this study was to identify the genome and perform phylogenetic analysis of respiratory viruses that are transmitted among ruminants that cohabit in an intensive production unit in Mexico City.
Material and methods
10 goats, 10 sheep and 10 cattle living together in an intensive production unit in Mexico City were used. Three samplings were carried out, the first was in winter, the second in summer and the third in winter. The polymerase chain reaction test with and without retro-transcription was performed to amplify a fragment of the genome of these three viral agents, the test product was purified and sent for sequencing to perform genetic characterization and phylogenetic inferences.
Results
The results confirmed that the co-habitation between different species predisposes to the transmission of these three viral agents and that there are genetic modifications in them that facilitate their adaptation to different hosts, in addition it is confirmed that in the case of viruses with respiratory tropism the season winter is a major factor and with respect to BDV, the time of year has no implication on its presentation.
Conclusions
These results allow us to establish the bases of the genetic characteristics of the viral agents that circulate in the ruminant production units in Mexico to allow us to establish preventive and biosecurity measures in the production units to prevent productive and economic decreases to the producers. Financing FONSEC SADER-CONACYT 2017-06-292826.

1340 - Molecular identification of BHV-1 and BVDV in two important dairy cattle producing areas in México

Author: Jose Francisco Rivera Benitez, Jazmín De La Luz, David Oseguera

Objectives
The objective of the present study was to identify the frequency of detection of bovine viral diarrhea virus (BVDV) and bovine herpesvirus type 1 (BHV-1), which causes infectious bovine rhinotracheitis (IBR).
Material and methods
A total of 9,276 samples of dairy cattle from the states of Jalisco and Guanajuato were analyzed. A real-time RT-PCR test was used for the identification of BVDV genotype 1 and 2 and an endpoint PCR test for the detection of BHV-1.
Results
108 (21.4%) positive samples were detected in the molecular test for BVDV1 and 44 (8.7%) positive samples for BVDV2, the year in which the highest positivity was identified was 2019 (66/13.1%), the state that obtained the highest proportion in the number of positives was Guanajuato (122/256, 47.6%). For BHV-1, the year with the highest detection was 2017 in the state of Guanajuato (19/3.7%) and in the state of Jalisco, 60 (24.2%) positive samples of 247 analyzed were detected in 2019. Results were validated of positive controls using a real-time PCR test.
Conclusions
With the results obtained, the frequency in the detection of both viral agents was identified. This information must be considered for the implementation of vaccination, diagnosis and control programs of these diseases in the national herd, with the aim of improving the health of the production units and avoiding associated economic losses.

1356 - Screening and comparative analysis of Vero cell clones for high-titer production of bovine respiratory syncytial virus (BRSV)

Author: Chieko Kuwahara, Chihiro Ueno, Shota Kondo, Shizuka Hirose, Kittipong Thanasaksiri

Objectives
Bovine respiratory syncytial virus (BRSV) is a major cause of lower respiratory tract disorders in calves. Isolation of the virus from clinical samples and its propagation can be performed using Vero cells. Vero cell sublines have shown different ability to replicate other viral species. In this study, various clones of Vero cells were isolated from its original and study of their susceptibility against BRSV isolates in Japan was conducted.
Material and methods
Limiting dilution cloning was used for isolation of single cell clones. Comparison of the growth performance of each clone and investigation of their ability for viral proliferation were carried out.
Results
Among 24 newly isolated clones, only a clone named Vero-CL7 had a fast growth rate and showed high robust viral propagation. No difference in susceptibility between original Vero cells and Vero-CL7 was observed. More than $10^{8.0}$ $TCID_{50}$/ml of BRSV was obtained within 6 days after inoculation into the new Vero cell clone.
Conclusions
Single cell cloning of Vero cells was successful and the highly permissive clone, Vero-CL7, was generated. The clone could produce high titers of BRSV regardless of viral isolates, indicating the usefulness of this clone for vaccine production. Comparative analysis of Vero cell clones will be further investigated and discussed for better understanding of cell proliferation and BRSV propagation. Optimization of cell culture condition will also be conducted to improve the stability of the cells for large scale viral production.

1359 - Comparative response of two single dose vaccines for controlling neonatal calf diarrhea in beef cattle

Author: L Elvira, PJ Bejar, P Piñeiro, M Vicente, L Fraile

Objectives

Neonatal calf diarrhea (NCD) is a leading cause of morbidity and mortality of beef calves in their first month of life (Smith, 2012; Naylor, 2009). resulting in growth retardation and increased the risk of pneumonia. Moreover, NCD has a negative impact on farm profitability, animal well-being, and increases antimicrobials use (Uter Maier et al., 2022).

Four of the most prevalent agents involved in NCD are Escherichia coli (ETEC), rotavirus (BoRV), coronavirus (BoCV) and C.parvum. Related to preventive strategies, a positive impact of vaccination of dams during the last third of pregnancy to protect passively the calves through colostrum has been shown (Recca et al., 2003). However, González et al. (2022) showed how the specific immune response after vaccination against NCD differs between apparent similar vaccines in dairy cows.

The objective of this study was to compare the results of two commercial vaccines for controlling neonatal calf diarrhea with a single dose registration at primary vaccination monitoring the immune response in beef dams and their calves.

Material and methods

The blinded and randomized study was performed in a two Spanish beef farms located in the NorthWest of Spain. In total 75 beef dams not previously vaccinated against NCD were randomly allocated to one of three study groups two months prior calving: a negative control group without vaccination and two vaccinated groups with two different vaccines (vaccine A: Bovilis Rotavec® Corona and vaccine B: Bovisan® Diar / Bovigen®). Calves sucked at their own dams and no differences in management occurred by sex.

To monitor the immune response, the concentration of specific antibodies against the three pathogens included in the vaccine (*E. coli*, rotavirus and coronavirus) was quantified in the serum and colostrum of the dams, and their calf's serum. For this, commercial ELISA tests (BIO K126 Bovine Rotavirus, BIO K295 *E. coli* k99 and BIO K392 Coronavirus bovine) were used.

All statistical analyses were carried out in a blinded way using SAS V.9.1.3 (SAS institute Inc., Cary, NC, USA). For all analyses, the individual dam or calf was used as the experimental unit. The significance level was set at 0.05 with statistical tendencies reported when p-value < 0.10. As baseline, homogeneity analysis and a multivariable model analysis were performed.

Results

A total of 37 cows calving between 3 weeks and 3 months after vaccination were followed up until the end of the trial: 10 in the vaccine A group; 15 vaccine B group and 12 non-vaccinated. Direct statistical analysis showed that colostrum of dams of Vaccine A group had significantly higher levels of antibodies against BoCV, BoRV and ETEC ($P<0.05$). Later, the multivariable analysis revealed the main impact of vaccine group for colostrum ETEC; and its main importance for BoCV and BoRV, as shown by a prediction formula (IE: Colostrum BoCV = 35.9 +0.46*TPV+ Group (Vaccine A: 21,27; Vaccine B: -3,45 and Control -17,9) + -0,06 ICV.

Moreover, the calves of dams from Vaccine A group had significantly higher levels of antibodies against ETEC compared to Vaccine B (93.4 vs 65.3% for Vaccine A and B, respectively) ($P<0.05$).

Conclusions

The results of the study showed the positive impact of vaccinating beef pregnant cows increasing the specific immune response enhancing the expected protection of their calves against NCD. In the control group, the average level of colostrum antibodies was significantly lower than Vaccine A for the three pathogens, demonstrating the importance of dams' vaccination. As previously shown in dairy, also in beef cows a more complete and balanced specific immune response against the three pathogens was found in the group vaccinated with vaccine A (Rotavec® Corona).

1363 - Mucosal and Systemic Immune Responses Following Intranasal Vaccination With Either a Bivalent Modified-Live Bacterial Vaccine or a Vaccine Containing the Same Bacteria and Three Modified-Live Viruses.

Author: Philippe Griebel, Guy Boisclair, Karthic Rajamanickam

Objectives

Intranasal (IN) vaccination with modified-live viral (MLV) vaccines provides an effective strategy to capitalize on rapid development of the mucosal immune system in the upper respiratory tract (URT) of neonatal calves and avoid vaccine interference by maternal antibody. Questions remain, however, about the immunogenicity of IN modified-live (ML) bacterial vaccines, especially when bacteria in the vaccine are also members of the commensal microbiota that colonizes the URT of neonatal calves. Furthermore, a previous study demonstrated that IN vaccination of neonatal calves with a MLV vaccine can alter the local immune response to respiratory pathogens that are members of the URT microbiota.

This study was designed to evaluate immune responses to *Mannheimia haemolytica* (MH) and *Pasteurella multocida* (PM) when Holstein heifer calves received a single IN dose of a bivalent modified-live vaccine containing PM and MH (Once PMH®- IN; Once PMH) versus a multivalent MLV vaccine co-formulated with both PM and MH (Bovilis® Nasalgen® 3 PMH; N3-PMH). Vaccine immunogenicity was evaluated by measuring IgA responses in nasal secretions, serum IgG and blood lymphocyte responses to PM and MH antigens. Furthermore, we investigated whether IN vaccination altered either total bacterial abundance or the abundance of PM and MH in the neonatal URT.

Material and methods

Study population consisted of 7 to 13 day-old Holstein heifer calves fed colostrum and then randomly assigned to either of the two vaccine groups (N=31/group) or the placebo (vaccine diluent; N=31) group. All calves were BVDV negative, had total serum protein greater than 5.1 gm/dl, and no clinical signs of respiratory disease or diarrhea at the time of vaccination. Nasal secretions, blood, and deep nasopharyngeal swabs (DNPs) were collected immediately prior to vaccination and weekly throughout a 35 day post-vaccination period. Calves were also scored weekly for signs of respiratory disease and diarrhea.

Results

There was no significant difference in the clinical score for diarrhea and respiratory disease when comparing among treatment groups. Maternal IgG was present in calves from all groups for both viral and bacterial components of the two vaccines but neither IN vaccine altered the decay of serum IgG titres specific for bovine herpesvirus-1 (BHV-1) or MH. On day 35 post-vaccination, PM-specific serum IgG titres were significantly ($P< 0.05$) higher in the placebo versus either vaccine group. The placebo group developed IgA responses in nasal secretions to both PM and MH but both vaccine groups had numerically higher IgA responses to MH and significantly ($P<0.05$) higher IgA responses to PM. The N3-PMH group also had significantly ($P<0.05$) higher IgA responses to BHV-1 than either the placebo or Once-PMH group. Both the Once-PMH and N3-PMH groups had significantly ($P<0.05$) higher blood lymphocyte proliferative responses to MH and PM antigens when compared to the placebo group but there was a significant ($P<0.05$) age-dependent decrease in tumour necrosis factor (TNF)-alpha secretion by blood

mononuclear cells in response to MH- and PM-stimulation in all treatment groups. Calves in all treatment groups displayed an age-dependent increase in total bacterial density in the URT. This was associated with an age-dependent decrease in PM density and an age-dependent increase in MH density that were not significantly altered by either IN vaccine.

Conclusions

In this study a single IN vaccination with modified-live PM and MH, either alone or co-formulated with a multivalent MLV vaccine, enhanced IgA antibody responses in nasal secretions and induced T lymphocyte responses in blood to both PM and MH. Thus, the IN vaccines are immunogenic despite early colonization of the bovine URT by both PM and MH and vaccination did not alter the density of either of these opportunistic respiratory pathogens in the URT. IN vaccines had no significant effect on clinical disease but increasing PM-specific serum IgG titres in the placebo group provided indirect evidence the IN vaccines may reduce PM colonization of the lung. If the IN vaccines provided protection against respiratory infection this may be mediated by PM- and MH-specific T lymphocyte responses that did not include a pro-inflammatory component.

1384 - An observational study of the efficacy of an inactivated vaccine against S. uberis in two dairy herds in Canada

Author: Dan Shock , Dolors Giralt-Casellas , Isabel Barril Basil , Massimiliano Baratelli

Objectives

Streptococcus uberis is a major bovine mastitis pathogen, often implicated in recurrent mastitis cases that respond poorly to antimicrobial therapy. Given the potential for significant health, welfare, and economic consequences, it is vital that dairy farms focus their efforts on preventing *S. uberis* mastitis. In Canada there is just one licensed vaccine against *S. uberis* clinical mastitis. The objective of this study was to evaluate the performance of this novel vaccine in dairy farms with a high incidence of *S. uberis* mastitis.

Material and methods

Three dairy farms located in the province of Quebec (Canada) were recruited for the study in 2020-2021. These farms had a high incidence of *S. uberis* clinical mastitis during the preceding year. The farms vaccinated the entire herd with [UBAC®] (HIPRA) by applying 3 intramuscular doses separated by a month interval. Animals were then re-vaccinated 6 months later. The vaccine is composed of an adjuvanted subunit antigen of *S. uberis*. Data gathered for the study included monthly test-day data (Lactanet, Ste-Anne-de-Bellevue, Quebec) for individual cow somatic cell count (SCC), milk production, fat, and protein. In addition, all clinical mastitis cases and microbiological culture results were recorded within the herd management software. These data were collected for the periods of one year before and one year after completing the vaccination program (considered one month after administering the 3rd dose of the vaccine). Linear score was calculated as (ln (scc/100)/0.693417) + 3. New and chronic infections were calculated based on SCC dynamic and using 200.000 SCC as cut-off in multiparous cows and 150.000 SCC in heifers. These were analysed by Chi-square, linear regression model considering vaccination group as factor and DIM, lactation and month as co-variate. These tests were implemented in R v4.03 software.

Results

The data gathered from both farms were stratified as belonging to primiparous and multiparous cows because mastitis might be presented differently in these populations. In fact, primiparous cows in farm A did not show any *S. uberis* mastitis during the pre-vaccination period (n=100). In the same farm, a total of 144 multiparous cows were studied before and 180 after vaccination. *S. uberis* mastitis were reduced from 9.72 % to 2.38 % between the two studied periods ($p<0.05$). In addition, this subpopulation showed a significant improvement of the average milk production (35.66 Kg Vs 37.33 Kg; $p<0.05$) and the linear score (3.18 Vs 2.24; $p<0.05$). Based on the SCC dynamic, new infections were reduced from 12.92 % to 7.55 %; chronic infections were reduced from 15.16 % to 8.12 % ($p<0.05$).

Farm B showed a statistically significant ($p<0.05$) reduction of *S. uberis*-associated clinical mastitis in primiparous cows. These decreased from 26.92 % before vaccination (over a total of 26 primiparous cows) to none after vaccination (23 primiparous cows). Beside this, the effect was also statistically significant ($p<0.05$) in multiparous cows where the cases were reduced from 22.86 % (total multiparous cows =35) to 18.42 % (total multiparous cows =38). In addition, the linear score of multiparous cows showed

also a reduction (2.72 Vs 2.34; p=0.063) though the difference was not significant. Based on the SCC dynamic, new infections were reduced from 10.99% to 8.02 %; chronic infections were reduced from 13.74 % to 10.29 % (p<0.05).

Farm C showed presence of 9.71 % of multiparous cows with *S. uberis* clinical mastitis during the period before vaccination. Despite this, the farm was excluded from the study because many chronic infections were observed and biased the results. No improvements were observed in this farm.

Conclusions

During 2020-2021, vaccination with [UBAC®] led to an improvement in the health of the studied Canadian herds when compared to the previous period. Specifically, the clinical mastitis rates associated with *S. uberis* decreased significantly after vaccination. In some cases, an improvement of cow productivity was detected. These results reinforce the use of UBAC® as a part of a plan to prevent *S. uberis*-associated clinical mastitis in cows and heifers.

1385 - Major virulence factors (S-layer) for Campylobacter fetus are present in antigens currently used in commercial vaccines.

Author: Sofia Acquistapace, Pablo Alonzo Crosa, Manuela Cilintano, Florencia Sardi

Objectives

Campylobacter fetus (Cf) is a bacterial infection that affects cattle. It can be transmitted during natural service or through infected semen. Cf sometimes infects humans as an opportunistic pathogen. Cf infection in cattle refers to two distinct clinical conditions that are of veterinary significance. The first condition is caused by *Campylobacter fetus* subspecies *venerealis* (Cfv), which leads to enzootic infertility in cattle. The second condition is caused by *Campylobacter fetus* subsp. *fetus* (Cff), which is associated with sporadic abortion outbreaks in cattle and sheep. The Cf surface layer (S-layer) proteins are major virulence factors involved in abortion caused by Cff in sheep and mice models. Cf is classified into two serotypes (A and B) based on the lipopolysaccharide composition of the outer membrane of the bacteria. Cff presents both serotypes, and occasionally the combined serotype AB, whereas Cfv presents only serotype A. This study aimed to evaluate the presence and expression of the S-layer genes in Cf strains included in commercial vaccines (VIRBAC Uruguay).

Material and methods

Molecular characterization was done based on Multiplex PCR using 16S (ribosomal DNA 16S), gene cstA (a genetic marker of Cf), gene parA (Cfv plasmid protein) and gene VirB11 (associated with Cfv pathogenicity). In addition, we assessed the presence of virulence genes associated with surface array protein (sap) serotype A (sapA) and serotype B (sapB). S-layer proteins presence/expression were studied in live culture and inactivated antigen (used to formulate commercial vaccines) by Western blot using specific rabbit hyperimmune sera for the S-layer and MALDI-TOF to evaluate the protein identity.

Results

The 16S rRNA gene fragment sequences obtained of both strains were compared with the NCBI public database and confirm that both strains belong to the species Cf. Multiplex PCR results for cstA, parA, VirB11 genes, and PCR assays for S-layer genes differentiated the strains at subspecies level. Cff and Cfv carried sapB and sapA, respectively. In live culture and inactivated antigen the S-layer sapB was identified in Cff by MALDI-TOF and S-layer sapA was demonstrated in Cfv by Western Blot and MALDI-TOF.

Conclusions

The results of this study clearly demonstrate the presence of critical virulence factors in *Campylobacter fetus* strains used in VIRBAC Uruguay commercial vaccines. Such features have been shown to be relevant antigens in vaccines offering protection to various animal species. The clinical significance of these findings will be further explored in future studies.

1392 - Enzootic bovine leukosis in Holstein calves: an early control strategy

Author: Marina Maurente Berón, Natalia Olivero-Deibe, Martín Fraga, Alejo Menchaca, Caroline da Silva Silveira

Objectives

Enzootic bovine leukosis (EBL) is an infectious disease of cattle caused by bovine leukemia virus (BLV). Studies suggest that calves are an important source of virus shedding, as calves infected during the first week of life continue to increase their proviral load until 12 months of age and remain high for years. In Uruguay, a study of 30 commercial dairy farms reported a prevalence of 9% (49/542) of BLV-positive neonatal calves, distributed in 47% of the farms. In addition, in a pre-colostrum follow-up, they presented high provirus loads. Considering all the above, this study aimed to determine a management and early detection strategy for BLV focused on reducing the transmission of the virus during the calf-rearing period.

Material and methods

An observational and longitudinal study was carried out for two years (2022-2023) in a commercial dairy farm that raises Holstein cows, with continuous calving throughout the year, and a general BLV seroprevalence of 67.7%. Calves are raised collectively and are supplied with pasteurized colostrum and milk.

Two different strategies were followed for blood-sampling of calves; In 2022 sampling strategy one, consisted of sampling all the calves born at a single time without intervention in the dairy farm management. During sampling strategy two (in 2023), after-birth calves were immediately housed in individual cages for up to 7 days and were sampled weekly throughout the year.

Proviral load was determined by quantitative real-time PCR (qPCR) and all positive calves were removed from the dairy farm. The prevalence of VLB for the two sampling strategies and the difference between proportions were calculated using the X 2 test with the Stata 14.0 software. The prevalence of VLB for the two sampling strategies and the difference between proportions were calculated using the X^2 test with the Stata 14.0 software.

Results

With sampling strategy one (single sampling in the year), a total of 217 calves that were 1 day to 6 months old were sampled and 35 were positive by qPCR and were removed from the herd. During sampling strategy two (weekly sampling), nine calves out of 208 aged between 0 and 7 days tested positive and were removed from the dairy farm. The BLV prevalence rates for sampling strategies one and two were 16.1% ($CI_{95\%}$= 11.7% – 21.6%) and 4.3% ($CI_{95\%}$= 2.25% – 8.14%), respectively. There was a significantly lower prevalence using strategy two ($p < 0.001$).

Conclusions

Both sampling strategies were efficient for the early detection of BLV in calves. Separating the calves immediately after birth and detecting positive animals in the first days of life, had a statistically significant lower positivity. Although this strategy requires a high number of samples and handling of calves, it could be attractive and feasible for this productive sector. The positive features of this strategy are: 1) a low number of calves that will have to be eliminated and only negative calves will be raised; 2) decreased probability of viral transmission through direct contact; 3) economic

feasibility (lower expenses with the breeding and discarding of positive calves); 4) replacement with negative heifers, that avoid the increase of the incidence of BLV in this category. In this context, management and early diagnosis strategies focused on reducing the transmission of BLV could be of great help to reduce the prevalence of infection and be used in future disease control protocols in dairy herds.

1394 - Anamnestic immune response demonstration in bovines vaccinated with clostridial vaccine.

Author: Florencia Sardi, Pablo Alonzo Crosa, Sofia Acquistapace, Manuela Cilintano, Natasha Barrandeguy

Objectives

Clostridiosis are toxic-infectious diseases, caused by bacteria of the genus *Clostridium* that can affect different animal species. Vaccination is a common practice to prevent clinical presentations that can arise from clostridial infections, such as enteric, gangrenous, hepatic, and neurotoxic diseases. As a result of the antigen presentation process following infection or vaccination, the immune system generates an effector response with antibody-producing plasma cells and memory B cells. These cells are the basis of the anamnestic response and are responsible for generating a more rapid and efficient antibody control of the infection in a subsequent encounter with the pathogen. The main objective was to investigate the anamnestic immune response developed in calves which were vaccinated with a clostridial, commercial vaccine by evaluation of specific antibodies.

Material and methods

A total of 12 6-month-old, cross beef breed calves were enrolled and were kept in pasture with free access to water. These calves did not have previous vaccination history for clostridial diseases and were randomly allocated into two groups: 1) Group C11 (n=6), animals vaccinated with a polyvalent clostridial commercial vaccine CLOSTRISAN 11™ (VIRBAC, Uruguay, including *Clostridium chauvoei, C. sordellii, C. perfringens types A, B, C, D, C. septicum, C. novyi types B and D, C. botulinum types C and D*), 2) sentinel group (n=6). Animals in group C11 were given two 5 mL doses of vaccine subcutaneously on days 0 and 22. A booster shot was administered to all animals on day 350 per the manufacturer's recommendation. On the same dates, the sentinel group was inoculated with 5mL of sterile saline solution. Blood samples were collected on days 0, 22, 35, 48, 77, 158, 350, 357, 364, 371 and 387, centrifuged to obtain serum, and stored at -20°C until titration. Specific antibody response was evaluated for eight of the eleven valences included in the vaccine. Eight different analytical tools previously set up were used in order to quantify antibodies using an in-house indirect ELISA assay (*C. botulinum D, C. botulinum C, C. perfringens C and C. chauvoei*) and by cell culture serum neutralization in vitro assay for *C. perfringens D, C. septicum, C. sordellii and C. novyi B*. Standard antitoxins (NIBSC – London, UK) were used to evaluate the samples and express the results in IU (international units) for each valence. The antibody responses were compared using a multiple comparison two-way ANOVA to determine significant differences against each valence (p<0,05). GraphPad Prism v9.0 software was utilized for the purpose of generating graphs and conducting statistical analyses to determine significant differences.

Results

After the first two doses (day 0 and 22) the peak of specific antibodies were detected on day 35 post vaccination for all valences tested in C11 group. Following the peak, the antibody levels decreased for all valences until day 350, eventually reaching the level observed on day 0. No statistical differences were observed in the level of specific

antibodies in the sentinel group. A faster response was observed after the booster dose (day 350) for all valences evaluated. No statistical differences were observed in antibody titres induced between the peak of the antibody response on day 35 (13 days after first two doses) and day 357 (1 week after booster dose) for all valences evaluated.

Conclusions

The fast increase in specific antibody response observed for all antigens evaluated is indicative of the memory cell response induced after primary vaccination with CLOSTRISAN 11™ and reinforces the recommendation of yearly booster with this vaccine.

1395 - Assessment of the bovine antibody response to clostridial antigens in two commercial vaccines.

Author: Manuela Cilintano, Pablo Alonzo Crosa, Florencia Sardi, Sofia Acquistapace, Natasha Barrandeguy

Objectives

Clostridiosis are toxic-infectious diseases, caused by bacteria of the genus *Clostridium* that can affect different animal species. Vaccination is a widely used practice to prevent enteric, gangrenous, hepatic, and neurotoxic diseases following infection. The aim of this study was to evaluate the immunogenicity of CLOSTRISAN 11™ commercial vaccine in cattle and compare the antibody response with a leading polyvalent clostridial vaccine present in the Brazilian market.

Material and methods

A total of 18 6-month-old, cross beef breed calves were used, which were kept in pasture with free access to water. These calves did not have previous vaccination history for clostridial diseases and were randomly allocated into three groups: 1) Group C11 (n=6), animals vaccinated with a polyvalent clostridial commercial vaccines (Clostrisan 11™ (Virbac, Uruguay, including *Clostridium chauvoei, C. sordellii, C. perfringens types A, B, C, D, C. septicum, C. novyi types B, D, C. botulinum types C, D*), 2) Group BMV (n=6), animals vaccinated with another polyvalent vaccine marketed in Brazil (containing C. *chauvoei, C. sordellii, C. perfringens types B, C, D, C. septicum, C. novyi B, C. botulinum types C and D*), and 3) a sentinel group with 6 animals injected with saline (0.9% NaCl). Animals were vaccinated by subcutaneous route with 1 dose of vaccine (5 mL) according to manufacturer's recommendations or sterile saline solution on days 0 and 22. Blood samples were collected on days 0, 22, 35, 48, 77 and 158, centrifuged to obtain serum, and stored at -20°C until titration. Specific antibody response was evaluated for common antigens included in both commercial vaccines. Eight different analytical tools previously set up were used in order to quantify antibodies using an in-house indirect ELISA assay (*C. botulinum D, C. botulinum C, C. perfringens C and C. chauvoei*) and cell culture sero-neutralization in vitro assay proposed by others for C. *perfringens D, C. septicum, C. sordellii and C. novyi B*. Standard antitoxins (NIBSC – London, UK) were used to evaluate the samples and express the results in IU (international units) for each valence. The antibody responses were compared using a multiple comparison two-way ANOVA to determine significant differences against each valence (p<0,05). GraphPad Prism v9.0 software was utilized for the purpose of generating graphs and conducting statistical analyses to determine significant differences.

Results

The antibody levels induced for commercial vaccines were significantly higher for all valences on day 35 compared with the placebo group, as was expected. Statistical analysis indicated that C. *sordellii, C. chauvoei, C. perfringens type C,* and C. *septicum* valences elicited a significantly higher immune response in C11 group compared to the same valences in BMV-vaccinated animals. Whereas the other valences (C. *botulinum types D, C, C. novyi B,* and C. *perfringens* type D), no statistical differences were detected between commercial vaccines.

Conclusions

Strong immune responses were observed for all vaccine components in both commercial vaccines. However, animals vaccinated with CLOSTRISAN 11™ showed a higher immune response to four valences (*C. sordellii*, *C. chauvoei*, *C. perfringens* type C, and *C. septicum*) compared to those vaccinated with the leading vaccine in the Brazilian market. Although the biological significance of this finding requires further investigation, it suggests that CLOSTRISAN 11 may be more effective against these four pathogens.

1408 - Targeted surveillance for bovine spongiform encephalopathy in cattle

Author: Timm Konold

Objectives

The World Organisation for Animal Health (WOAH) has recently modified the chapters of the Terrestrial Animal Health Code relating to Bovine Spongiform Encephalopathy (BSE) in cattle to concentrate on targeted BSE surveillance to support assessment of a country's BSE risk status. This requires targeting cattle with clinical signs compatible with BSE, such as cattle with suspicious nervous signs identified on farms or abattoirs, cattle unable to rise or walk without assistance and fallen stock where the clinical history cannot be attributed to other common causes. However, the dramatic decline in the number of BSE cases linked to the consumption of contaminated feed (classical BSE) has led to a lack of cases, and the majority will have never seen a BSE case, which makes targeted surveillance a challenge.

The objective of this study was to retrospectively use the clinical sign data of clinical suspects collected during the BSE epidemic in Great Britain to aid in the clinical diagnosis. In addition, veterinary officers involved in case selection during the epidemic were asked to provide a definition of these particular clinical signs that may be useful for setting up a targeted surveillance program.

Material and methods

The study included data from 69,784 cattle examined once between 1993 and 2005, 68,404 (99.7%) were diagnosed as BSE cases by postmortem diagnostic tests. The 30 assessed clinical signs were: apprehension, hypersensitivity to touch and sound, maniacal and panic stricken behaviour, temperament change, abnormal head carriage and ear angle, abnormal behaviour, head shyness, licking flank and nose, kicking, reluctance to go through doorways, head pressing and rubbing, teeth grinding, blindness, circling, hindleg and foreleg ataxia, falling, paresis, knuckling of fetlocks, tremor, recumbency, loss of weight, condition and milk yield. A feature selection model was used to identify the best predictors for a clinical diagnosis of BSE based on statistical significance.

A questionnaire was sent to 21 veterinary officers (VOs) to select the most appropriate definition for each clinical sign, with a free text field for alternative or additional definitions.

Results

Out of the best ten predictors, six were more indicative of BSE: hypersensitivity to sound, ear twitching, hypersensitivity to touch, reluctance to go through doorways, teeth grinding and apprehension whilst four were more indicative of another disease: blindness, circling, maniacal behaviour and recumbency. The classical sign combination of apprehension (including reluctance to go through doorways), hypersensitivity (including kicking) and ataxia was recorded in 55,367 (72%) BSE cases compared to 973 (50%) BSE suspects that were not confirmed as BSE cases.

The sign definitions that the majority of VOs agreed on were: "startle or flinch to sudden unexpected environmental noise" for hypersensitivity to sound, "repeated ear twitching" for ear twitching, "over-reactivity to flies with frequent tail swishing and head tossing" for hypersensitivity to touch, "stopping frequently on the way to the parlour or

through doorways" for reluctance to go through doorways, "teeth grinding when idling" for teeth grinding and "staring eyes or anxious look" for apprehension, "walking into objects or walls but eyes appear normal" for blindness, "walking aimlessly around in circles" for circling, "charging people" for maniacal behaviour and "animal is currently down" for recumbency.

Conclusions

Not unexpectedly from previous publications, hypersensitivity and apprehension were good BSE sign markers, although ataxia was not in the top 10 predicting signs, whilst circling, blindness, maniacal behaviour and recumbency were more associated with BSE suspects that were not confirmed.

Startle to sudden auditory stimuli appears to be a good clinical predictor, which can be easily tested. A detailed neurological examination may still represent the best method for a clinical diagnosis but requires more specialist knowledge, whereas assessment of the presence or absence of particular clinical signs associated with a neurological disease in cattle may be more useful for general veterinary practitioners or farmers. However, clinical signs should be adequately defined to avoid misinterpretation.

1410 - Seroprevalence and risk factors associated with BRSV in Dutch dairy calves

Author: Ruurd Jorritsma Jorritsma, Tine van Werven, Maurice van den Hoven, Rineke de Jong

Objectives
Bovine respiratory syncytial virus (BRSV) is an infectious disease affecting young calves. This study aimed to determine the seroprevalence of BRSV on dairy farms at the herd level in the Netherlands and evaluate the risk factors associated with the occurrence of the disease in the winter of 2021-2022.
Material and methods
We collected five blood samples from animals between 240 and 365 days old on farms that were either participating in the Dutch BVDV control and monitor program or had recently respiratory problems in youngstock. In addition, we interviewed the farmers by phone with questions about housing and management.

Results
We successfully enrolled 139 farms in the study. On 113 farms, we found at least one calf with BRSV antibodies (81,3%, CI 74,8 -87,8). Overall, 516 out of the 691 calves were found positive (74,7%, CI 71,4-78,0). A total of 86 farmers were interviewed during a telephone survey. We found that a higher ratio of youngstock/cows and grazing of calves were significantly associated with seropositivity for BRSV. In addition, moving calves for a second time to another group above the age of 90 days and using groups of more than 5 calves during rearing were also associated with seropositivity for BRSV
Conclusions
We concluded that BRSV infections are very common in youngstock on Dutch dairy herds. Our results suggest that calves are more frequently infected when they have more contacts with other calves and adult cows.

1414 - Respiratory illness in young and adult cattle caused by BVDV-2b in singular and mixed bacterial infection in a dairy herd.

Author: ALICE ALFIERI, Juliana Fritzen, Carolina Yasumitsu, Isabela Silva, Michele Lunardi, Juliane Ribeiro, Alais Dall Agnol, AMAURI ALFIERI

Objectives

Bovine respiratory disease (BRD) is a common health problem in dairy cattle herds worldwide. BRD is a multifactorial disease that affects young (calves and heifers) and adult (cows) animals. Aspects related to animals (age, nutrition, immunity) and management (animal density and biosecurity practices) may predispose to the occurrence of BRD in dairy cattle herds. However, infections of the upper and lower respiratory tract caused by viruses and bacteria, which occur in both singular and mixed infections, are the major causes of the clinical signs of BRD. The definitive diagnosis of BRD is complex as the etiology involves the association of several predisposing and determining factors. This report describes the etiology of an outbreak of BRD in a dairy cattle herd in the central-eastern region, Paraná State, southern Brazil.

Material and methods

The BRD outbreak occurred in young (calves and heifers) and adult (cows) Holstein cattle. Nine biological samples, consisting of five lung samples from two cows and three suckling calves and four nasal swab samples from heifers, were used to carry out the etiological diagnosis. The nucleic acid extracted from lung fragments and nasal swabs were submitted to amplification by PCR and RT-PCR assays for partial amplification of genes of five viruses (bovine viral diarrhea virus - BVDV, bovine alphaherpesvirus 1 - BoAHV-1, bovine respiratory syncytial virus - BRSV, bovine parainfluenza virus 3 - BPIV-3, and bovine coronavirus - BCoV) and four bacteria *Mycoplasma bovis, Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*) involved in the etiology of BRD.

Results

All nine biological samples from animals with BRD evaluated were negative for BoAHV-1, BRSV, BPIV-3, BCoV, and *Histophilus somni*. Therefore, the participation of these microorganisms in the etiology of the BRD outbreak investigated can be ruled out. In fragments of lung tissue from a cow and three calves, it was possible to identify the presence of BVDV and *M. bovis*. These two pathogens of the lower respiratory tract of cattle were present in singular and mixed infections. BVDV also was identified in two nasal swabs, one as a single etiological agent and the other in association with two bacteria (*P. multocida* and *M. haemolytica*). The phylogenetic analysis carried out in the nucleotide sequence of the 5'UTR region and N^{pro} gene of the BVDV amplicons demonstrated that the BVDV field strains of this BRD outbreak belong to subgenotype 2b.

Conclusions

To our knowledge, this is the first report of the BVDV-2b involved in the etiology of BRD in Brazil. Finally, it is needed to highlight that the cattle came from an open dairy cattle herd with regular vaccination for BVDV-1 and -2. We concluded that in open dairy cattle herds that incorporate heifers or cows of neighbors into the herd for maintenance of milk production, only a vaccination program against BVDV-1 and -2 is not enough to control the infection. Additional internal and external biosecurity measures are essential

to prevent different subgenotypes of BVDV-1 or -2 from being introduced and spread across the animal categories (suckling and weaned calves, heifers, and cows) of a dairy cattle herd.

1415 - Outbreak of persistently infected heifer calves with bovine viral diarrhea virus subgenotypes 1b and 1d in a BVDV-vaccinated open dairy herd.

Author: AMAURI ALFIERI , Juliana Fritzen, Natalia Zaparoli, Juliane Ribeiro, Michele Lunardi, Alais Dall Agnol, ALICE ALFIERI

Objectives

Bovine viral diarrhea virus (BVDV) is an important pathogen in cattle because it can cause severe economic losses in the beef and dairy industries and decrease fertility and milk production worldwide. BVDV infection can result in subclinical infections or a wide range of distinct clinical signs, such as diarrhea, respiratory distress, and reproductive dysfunctions, including infertility, abortion, malformations, and persistently infected (PI) offspring. Reproductive failures are the most important impact caused by BVDV infections and occur according to the gestation period of infection, biotype (cytopathic - cp and non-cytopathic - ncp) of the virus, and viral strain. In fetal infection by BVDV ncp strains between 40 and 120 days of gestation the fetal immune system is still immature and incapable of differentiating between viral proteins and self-proteins. These conditions can lead to the birth of immunotolerant and PI calves which is the main event in the epidemiological chain of the infection. This report describes the birth of 99 BVDV-PI heifer calves, born within 52 days in a regularly BVDV-1 and BVDV-2 vaccinated Brazilian dairy cattle herd, as well as the subgenotypes of the infecting BVDV field strains.

Material and methods

The study was developed in a high-yielding open dairy cattle herd with approximately 1,500 lactating Holstein cows and an average milk production of 29.2 liters/cow/day. The farm is located in the central-eastern region, Paraná State, southern Brazil, that frequently acquired heifers from neighboring for replacement. The farm monitors the birth of PI calves by screening all calves born with an ELISA test (IDEXX BVDV Ag/Serum Plus, Westbrook, Me, USA) for BVDV-antigen detection, according to the manufacturer's instructions. All calves aged 1 to 7 days of life were evaluated. In positive and suspect results the ELISA test was repeated when the calves were close to one month old. To evaluate the predominant BVDV species and subgenotype in this outbreak, whole blood samples were collected from 31 calves born during the study period. All samples were submitted to the RT-PCR assay for the partial amplification of the BVDV 5'UTR region and the N^{pro} gene. All amplicons obtained were submitted to nucleotide sequencing.

Results

From February to March 2021, were evaluated 294 heifer calves. Of these, 99 (33.7%) were positive in two ELISA tests and were considered PI calves. The phylogenetic analyses performed on whole blood samples from 31 heifer calves allowed the identification of BVDV-1b and BVDV-1d in 16 and 13 heifer calves, respectively. In two calves, due to the quality of the nucleotide sequence, was not possible to determine the BVDV-1 subgenotype.

Conclusions

Detection of PI animals and monitoring of the circulating BVDV subgenotypes are central to disease control. The study showed that only regular BVD vaccination may not be enough to prevent the BVDV infection in high-yielding open dairy cattle herds. Other biosecurity actions such as clinical examination, quarantine, vaccination, and testing of acquired animals must be adopted to avoid the purchase of cattle with acute infections by BVDV or even BVDV-PI, which causes a break in the health profile of the herd and economic losses.

Financial support: National Institute of Science and Technology for Dairy Production Chain (INCT–LEITE), CNPq, CAPES, and Fundação Araucária (FAP/PR).

1416 - Neglected bacterial infections associated to bovine respiratory disease in lactating cows from high yielding dairy cattle herds.

Author: ALICE ALFIERI, Rodrigo Massi, Michele Lunardi, Juliane Ribeiro, Alais Dall Agnol, AMAURI ALFIERI

Objectives

The bovine respiratory disease (BRD) complex is a multietiological and multifactorial syndrome that occurs due to an imbalance in the immune system of animals and external factors that favor the disease. Viruses and bacteria such as bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCoV), bovine viral diarrhea virus (BVDV), bovine alphaherpesvirus 1 (BoAHV-1), bovine parainfluenza virus 3 (BPIV-3), *Mycoplasma bovis, Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni* may be involved in the etiology of BRD in both single and, more frequently, in mixed infections. Due to an increase in morbidity and mortality rates in cattle herds, BRD is responsible for significant economic losses resulting from reduced productivity of dairy herds. This study aimed to investigate the main infectious agents associated with acute bovine respiratory disease outbreaks in adult dairy cows in southern Brazil.

Material and methods

This study was carried out on two dairy farms located in the city of Carambeí (24 ° 55' 04" S, 50 ° 05' 49" W) in the central-eastern mesoregion of Parana State, Brazil. The distance between the farms was approximately 30 km. Furthermore, no cattle exchange between the two herds had been reported. Herds A and B were composed of 280 and 450 cows, respectively. They had a daily production of 30 and 38 liters of milk per cow, respectively, and produced 8,400 and 17,100 liters of milk/day, respectively. Both farms with Holstein cows are considered closed dairy cattle herds. During the winter season, an outbreak of acute BRD occurred in the lactating cows on the two farms. The morbidity rates in herds A and B were 6.6% and 8%, respectively, targeting cows at peak lactation. On both farms, no cow deaths were reported.

Results

To determine the etiology of the clinical cases of acute respiratory disease, nasal swab samples (*n*=28) were collected from cows with clinical signs of BRD and evaluated using PCR and RT-PCR assays for nucleic acid detection of the main BRD etiological agents, including *M. bovis*, *M. haemolytica*, *P. multocida*, *H. somni*, BRSV, BCoV, BVDV, BoAHV-1, and BPIV-3. In both herds (A and B), were not identified nucleic acids of some of the main microorganisms involved in the etiology of BRD evaluated in this survey, including the viral agents such as BoAHV-1, BVDV, BRSV, BPIV-3, and BCoV, and bacteria (M. haemolytica). Only three microorganisms (*M. bovis*, *H. somni*, and *P. multocida*) were identified in single and mixed infections. We concluded that 40.0% of the cows were infected with *M. bovis* and 75.0% with *H. somni* in herd A. Considering both single and mixed infections, the analyses performed in herd B showed that 87.5%, 25.0%, and 50.0% of the cows were infected with *M. bovis*, *H. somni*, and *P. multocida*, respectively.

Conclusions

M. bovis and *H. somni* are considered fastidious bacteria. With this, the laboratory diagnosis is frequently neglected. With this, most clinical cases of mycoplasmosis and histophilosis in cattle remain undiagnosed. This study demonstrates the importance of

M. bovis and *H. somni* infections in adult cows with BRD. These results highlight the importance of including these bacteria in the group of the etiological agents responsible for the occurrence of BRD in cattle, especially in adult cows with unfavorable immunological conditions, such as recent calving and peak lactation.

Financial support: National Institute of Science and Technology for Dairy Production Chain (INCT–LEITE), CNPq, CAPES, and Fundação Araucária (FAP/PR).

1424 - Development of a recombinant targeted vaccine against BVDV type 1a, 1b and Type 2.

Author: Romina Cardona, Valentina Carrettoni, Lucía Rocha, José Angel Escribano, Demian Bellido, Viviana Parreño, Andrés Wigdorovitz

Objectives

The objective of the present study is to develop and evaluate in a guinea pigs model a targeted vaccine against the predominant genotypes of Bovine Viral Diarrhea Virus, genotype 1, sub-genotypes 1a and 1b, and genotype 2.

Material and methods

To target the immune system the immunodominant protein of Bovine Viral Diarrhea Virus (BVDV), the E2 glycoprotein, is fused to APCH, which is a single-chain antibody that targets the MHC-II molecule present in the antigen presenting cells, resulting in the APCH-E2 fusion protein.

To formulate the vaccine three different proteins were cloned and expressed in the baculovirus expression system: APCH-E2 1a, APCH -E2 1b and APCH-E2 T2. Each E2 protein represents a genotype or sub-genotype of BVDV.

Protein identity was verified through Western Blot and protein were quantified by Dot-Blot.

Expression was optimized in 10 L wave bioreactors by analyzing different cell count, virus concentrations, harvest times, rocking speed, among other parameters.

Vaccine containing the three antigen was formulated with oil-adjuvant and a dose response test was performed in the guinea pig potency tests. Animals (5 per group) were vaccinated on day 0 and 21 with 1/5 of the bovine doses (3 ml). Serum samples were collected on days 30 and 60 post first inoculation. Vaccine immune response against the three genotypes was determined by sero-neutralization assay.

Results

Optimal infection dilution was determined in a 50 ml cell culture volume, and the scaling up to 10 L in wave bioreactors was achieved. Cultures were infected with a MOI of 0.03 and proteins were harvested 7 days post infection.

All three APCH-E2 proteins were successfully expressed, confirmed by Western Blot and quantified by Dot-Blot: 10 µg/ml micrograms for APCH 1a, 2.5 µg/ml micrograms for APCH-E2 1b and 5 µg/ml micrograms for APCH -E2 T2 were obtained.

The vaccine incorporating the three antigens demonstrated a highly satisfactory rating for APCH-E2 1a and APCH-E2 1b and a satisfactory rating for APCH-E2 T2, according to the national regulatory authority (SENASA).

Conclusions

Three different variants of the APCH-E2 antigens were generated, expressed, quantified and mixed together to formulate a new targeted multi-genotype vaccine against BVDV.

The nobel vaccine was evaluated in the guinea pig model with results that overpass the threshold imposed by the national regulatory authorities (SENASA).

Development of experimental batches and submission of the registration dossier to SENASA are underway, suggesting a forthcoming step towards the commercial availability of the vaccine.

The development of a new targeted vaccine against the more prevalent strains of BVDV represents a significant advancement in the BVDV vaccine, with substantial benefits for animal health and the prosperity of the livestock industry since targeted vaccine combines the potency of the live attenuated vaccines with the safety of the inactivated vaccines.

1445 - Prevalence of Different Presentation Forms of Bovine Enzootic Leukosis in Dairy Farms in the Province of Lima, Peru

Author: Rocío Silvia Sandoval Monzón, Luis Felipe Ruiz García

Objectives

To determine the prevalence of different presentation forms of the disease caused by bovine enzootic leukosis virus in dairy farms in the province of Lima, Peru.

Material and methods

The study was conducted in the province of Lima between January 2021 and February 2022, using samples from 16 dairy farms. A proportional random sampling was performed considering five age strata, and a total of 354 dairy cows were analyzed. The study included farms with more than 20 cattle heads that supplied their milk production to the dairy industry.

The methodology focused on blood sampling from animals older than 6 months, excluding pregnant cows over 8 months and recently calved cows up to two months postpartum (Rama et al., 2012). The study design classified animals according to age and different forms of bovine enzootic leukosis presentation. Seroprevalence and the prevalence of different presentation forms were calculated according to age group, and significant differences between groups were assessed. Blood samples were collected through puncture of the tail vein, and bovine leukosis diagnosis was performed using the INGEZIM BLV COMPAC 2.0 kit. Hematological and clinical diagnostics were also applied to assess lymphocytosis and lymphadenopathy, respectively. Statistical analysis included the chi-square test to determine possible significant differences between age groups.

Results

The study reveals that the seropositivity prevalence for bovine leukosis virus (BLV) in Lima province farms was 52.26%. A significant variation in prevalence was observed among age groups, with higher rates in cows aged 5 to 7 years (80.33%) and over 7 years (80%). Lymphocytosis prevalence was 14.69%, higher in the 5 to 7 years group (21.31%), and high lymphocytosis prevalence was 7.63%, more common in the 5 to 7 years group (13.11%). Tumor prevalence associated with bovine leukosis was 1.41%, higher in the over 7 years group (3.33%). The frequency of lymphocytosis in seropositive animals was 28.11%, while the frequency of tumors in seropositive animals was 2.7%, and the frequency of high and very high lymphocytosis in seropositive animals was 13.51% and 14.59%, respectively. In terms of statistical significance, seropositivity prevalence was significantly different among age groups ($p < 0.001$). Other variables did not show significant differences between age groups. It is noteworthy that the highest prevalence is recorded in the age group of 5 to 7 years and the over 7 years group, while the lowest prevalence is in the age group of 0.5 to 1 year. It was also found that the population of animals with very high lymphocytosis was 14%. This population represents the one with the highest proviral load, i.e., the population with the highest risk of virus transmission in the herd (Juliarena et al., 2007, Mekata et al., 2018).

Conclusions:

The study reveals a significant prevalence of bovine leukosis virus (BLV) in Lima province farms, reaching 52.26% seropositivity, 14.69% lymphocytosis, and 1.41% tumors associated with BLV.

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Juliarena, M. A., Poli, M., Sala, L., Ceriani, C., Gutierrez, S., Dolcini, G., Rodríguez, E.M., Dubra, C., & Esteban, E. N. (2008). Association of BLV infection profiles with alleles of the BoLA-DRB3. 2 gene. *Animal genetics*, *39*(4), 432-438.

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1446 - Efficacy of the Detection and Segregation Strategy for Bovines with Lymphocytosis in Reducing the Transmission of Bovine Leukosis Virus in Dairy Farms

Author: Rocío Silvia Sandoval Monzón, Luis Felipe Ruiz García

Objectives

To evaluate the efficacy of the detection and segregation strategy for bovines with lymphocytosis in reducing the transmission of bovine leukosis virus in a dairy farm.

Material and methods

This study was conducted in two dairy farms in the province of Lima, during the period from May 2020 to May 2021. We worked with 150 bovines seronegative for bovine leukosis virus (BLV) over 6 months old, monitoring them for 6 months. Serum samples were collected at the beginning of the study and at 6 months. A quasi-experimental design was employed, dividing animals into two groups based on the farm they belonged to: Control Group (bovines raised under normal conditions) and Intervention Group (bovines raised with prior detection and segregation of animals with lymphocytosis and lymphosarcoma). The study consisted of three stages: identification of animals with lymphocytosis and lymphosarcoma, determination of cumulative incidence in both groups, and comparison of cumulative incidence between them. Serological and complete blood count tests were conducted, using the ELISA test to diagnose BLV seropositivity and a leukocyte count to identify animals with lymphocytosis. Statistical analysis was performed using IBM SPSS Statistics 22, calculating cumulative incidence and conducting Chi-square tests.

Results

In the control group herd, 81 seronegative animals were found at the beginning of the monitoring period out of a total of 157 animals, resulting in a prevalence in that herd of 48%. In the intervened group herd, 86 seronegative animals were found out of a total of 167 animals, resulting in a prevalence in that herd of 49%. In the intervened herd, 11 seropositive animals with lymphocytosis exceeding 10,000 lymphocytes/µL were eliminated, with a count range between 11,060 to 20,320 lymphocytes/µL. After the 6-month period, all seronegative animals were reassessed for seroconversion. It was found that in the control group, 22 out of 81 animals became seropositive, resulting in a cumulative incidence of 35%. Meanwhile, in the intervened group, only 10 out of 86 animals became seropositive, resulting in a cumulative incidence of 13%. Analyzing the results by age group, it was found that in the control herd, animals aged 6 months to one year had an incidence of 13%, while in the intervened herd, the incidence in that age group was 0%. In the age group of 1 to 3 years, animals in the control group had an incidence of 33%, while the intervened group had a cumulative incidence of 12%. In the age group of 3 years and older, the control group had a cumulative incidence of 100%, while the intervened group had an incidence of 15%. The results of this study demonstrated that the strategy of detecting and segregating bovines with lymphocytosis reduces the transmission of bovine leukosis virus (Juliarena et al., 2007, Mekata et al., 2018).

Conclusions:

These results provide conclusive evidence that the strategy of detecting and segregating bovines with lymphocytosis effectively reduces the transmission of bovine leukosis virus in the intervened herd.

References:

Juliarena, M. A., Poli, M., Sala, L., Ceriani, C., Gutierrez, S., Dolcini, G., Rodríguez, E.M., Dubra, C., & Esteban, E. N. (2008). Association of BLVinfection profiles with alleles ofthe BoLA-DRB3. 2 gene. Animal genetics, 39(4), 432-438.

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1455 - CONTAGIOUS BOVINE PLEUROPNEUMONIA – CURRENT DATA FROM POLAND

Author: Katarzyna Dudek, Dariusz BEDNAREK

Objectives

Mycoplasma mycoides subsp. *mycoides* (*Mmm)* is an etiological agent of contagious bovine pleuropneumonia (CBPP), the World Organisation for Animal Health (WOAH) - listed disease. To control infection with *Mmm*, bovine serum samples from various regions of Poland are tested annually. The study aimed to demonstrate the evaluation of the prevalence of *Mmm* infections in Poland in 2022 based on serological screening of cattle.

Material and methods

A total of 799 sera were collected in 2022 from cattle orginating from five regions of Poland, i.e. eastern (240 sera), central (80 sera), southern (159 sera), north-western (160 sera) and northern (160 sera). The sera were analyzed using two commercially available methods intended for use in serological dignostics of CBPP: C-ELISA (IDEXX Contagious Bovine Pleuropneumonia, IDEXX Montpellier SAS, France) and a complement fixation test (CBPP Complement Fixation TEST Kit, CIRAD, France). Samples with positive or doubtful results in the first examination were re-analyzed using the same method.

Results

The first ELISA examination resulted in 35 positive sera. These samples originated from eastern (17 sera), central (7 sera), southern (3 sera), north-western (7 sera) and northern (one serum) regions of Poland. The remaining samples were negative. All positive samples were reduced to negative after the second examination using the same method. For comparison, 70 sera gave doubtful results in the first CFT examination, whereas the remaining 729 samples were negative. These samples originated from eastern (21 sera), southern (27 sera), north-western (14 sera) and northern (8 sera) regions of Poland. However, the second CFT examination resulted in all negative sera.

Conclusions

Despite several positive or doubtful results, they were not confirmed in subsequent studies using the same methods. False positive results are possible in the case of serological methods such as ELISA and CFT. It is associated with the possible occurrence of cross-reactions with other mycoplasma species. Further screening of cattle for CBPP in Poland is needed to assess the current epizootic situation in this field.

1456 - Whole Genome Sequencing of a wild-type Bovine alphaherpesvirus 1 (BoAHV-1) strain isolated in central Italy

Author: Stefano Petrini, Valentina Curini, Cecilia Righi, Barbara Secondini, Luana Fiorella Mincarelli, Massimo Ancora, Giulia Costantino, Michela Pela, Cesare Cammà, Francesco Feliziani

Objectives

Bovine alphaherpesvirus 1 (BoAHV-1), belongs to the family *Herpesviridae,* genus *Varicellovirus*, causes different clinical syndromes in cattle, such as infectious bovine rhinotracheitis (IBR). BoAHV-1 is widespread worldwide and causes significant economic losses on farms. However, in Italy, the genetic features of the virus are poorly understood. Therefore, this study aimed to characterize the genome of a wild-type BoAHV-1 isolated from a cattle herd with severe respiratory symptoms in central Italy.

Material and methods

During a disease outbreak in a beef cattle herd (consisting of 30 animals) in central Italy, we collected nasal swabs and blood samples from 12 animals with respiratory clinical symptoms. The cattle had not been vaccinated against bovine respiratory diseases and were located in a non-IBR-free area. Serum samples and nasal swabs were tested for BoAHV-1, Bovine respiratory syncytial virus (BRSV), Bovine parainfluenza-3 (BPI-3) virus, Bovine viral diarrhea virus (BVDV), *Mannheimia hemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis.* Serum samples were evaluated using commercial ELISA (IDEXX IBR gB X3 Ab; PRIMACHECK BRSV Ab; PRIMACHECK PI-3 Ab; IDEXX BVDV p80 Ab) and virus neutralization tests against BoAHV-1. The protocols used were those of the ELISA tests and those described in the OIE manual [1], respectively. Nasal swab samples were used for bacteriological and virological investigations. The first investigations were conducted using the protocols previously described by Carter and Coll. [2], while virus isolation was carried out using Madin-Darby bovine kidney (MDBK) cell cultures. The positive samples were confirmed using gB real-time PCR [1]. Subsequently, one of the three isolates was selected and identified as 16453/07 TN BoAHV-1, amplified in flasks of MDBK cells and ultracentrifuged at 16,000 rpm for 1.5 hours at 4°C. Later, the DNA was extracted as described by Petrini et al. [3] and sequenced using Illumina DNA Library Prep and the next-generation sequencing NexSeq2000 platform (Illumina Inc.). To obtain the whole genomic sequence (WGS) of BoAHV-1, bioinformatic analyzes were carried out on the GENPAT platform (https://genpat.izs. it/cmdbuild/ui/#login). A phylogenetic analysis was performed using the genome sequence of isolate 16453/07 TN together with 51 complete BoAHV-1 genomes (including those reported mainly from the USA along with those from China, India, and Australia) available in NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/) thorough the Molecular Evolutionary Genetics Analysis (MEGA) software (https://www.megasoftware.net/).

Results

Serological investigations evidenced positivity for BRSV (12/12 samples) and BPI-3 (11/12 samples) by ELISA tests. In addition, the Neutralizing Antibodies to BoAHV-1 were detected (9/11 samples). Virological results showed BoAHV-1 positivities on MDBK cells (3/12 samples). In addition, bacteriological investigations identified *Mycoplasma bovis* (4/12 samples). Using NGS sequencing of the DNA extracted from 16453/07 TN strain

(one of three isolated), 7,436,870 reads were retained from raw reads after quality control and trimming. Mapping of the trimmed reads against the reference BoHV-1 Cooper strain genome (GenBank Accession JX898220) produced a consensus sequence of 134,821 bp with an average vertical coverage of 1058 × and horizontal coverage of 99.5%. The complete genomic sequence obtained has been deposited in GenBank (accession Number: OR211605) and showed a nucleotide identity > 99% with all complete BoAHV-1 genomes type 1.1. available in NCBI, as also highlighted by phylogenetic analysis with MEGA software.

Conclusions

In this study, by WGS, the first complete sequence of BoAHV-1 circulating in Italy was obtained. Phylogenetic analysis underlined the low genetic variability of this virus, which has an identity nucleotide greater than 99, 2% with all complete genomes subtype 1.1 available in the NCBI, despite the geographical and temporal distance. The 16453/07 TN isolate is used by the National Reference Center for IBR to experimentally infect animals (calves/buffaloes) and test the safety and efficacy of different marker vaccines.

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1459 - The effect of colostrum supplementation on resolution of clinical signs and the fecal microbiota in calves with diarrhea caused by Cryptosporidium parvum

Author: Lisa Gamsjaeger , Karina M. Cirone, Steffany Schluessel, Mackenzie Campsall , Aydin Herik, Diego E. Gomez, Claire Windeyer, Eduardo Cobo, Saria Otani, Panagiotis Sapountzis, Antoine Dufour, Priyoshi Lahiri, Daniel Young

Objectives

Calves are highly susceptible to gastrointestinal infection with *Cryptosporidium parvum (C. parvum)*, which can result in watery diarrhea and eventually death or impaired development. With little to no effective therapeutics, investigation of alternative treatment approaches is warranted. Colostrum administered to calves after 24 of hours of age has been shown to reduce diarrhea in neonatal calves, and colostrum has been used to treat clinical Cryptosporidiosis in several species, including humans.

Objectives

The objectives were to determine the effect of bovine colostrum supplementation after the first day of life on resolution of clinical sign in calves experimentally infected with *C. parvum*, and the effect of both *C. parvum*infection and colostrum supplementation on the fecal microbiota in those calves.

Material and methods

A total of 25 newborn calves were randomly allocated into one of four groups: unchallenged (i.e., Sham) calves fed milk replacer (Sham/MR; n=5), unchallenged calves fed colostrum (Sham/C; n=6), *C. parvum* challenged calves fed milk replacer (*C. parvum*/MR; n=7), and *C. parvum* challenged calves fed colostrum (*C. parvum*/C; n=7). Calves in the *C. parvum*/MR and *C. parvum*/C groups were orally administered isolated peracetic acid-disinfected *C. parvum* oocysts (5×10^7 Iowa isolate from Dr. MW. Riggs, School of Animal and Comparative Biomedical Sciences, University of Arizona) in phosphate buffer solution (PBS) mixed with 25 ml of milk at 3–4 days of age, whereas the Sham groups received the same volume of just PBS and milk. Beginning at 2 days post challenge (dpc), calves in groups Sham/C and *C. parvum*/C were supplemented with 1L of bovine colostrum replacer once daily. Calves were examined daily and clinical parameters including heart rate, respiratory rate, body temperature as well as attitude, hydration status, ability to rise, appetite, and fecal consistency were recorded. The need for administration of oral electrolyte solution (OES; as determined by dehydration and attitude scores) was recorded. Fecal samples were collected on 0, 2, 4, and 6 dpc and on the first day of diarrhea to screen for other enteropathogens including *Escherichia coli (E. coli)* K99, *Salmonella*spp., and bovine rota- and coronavirus and for shotgun metagenomic sequencing. Clinical data was evaluated by repeated measures two-way analysis of variance (ANOVA). The number of OES treatments was compared by Fisher's exact test. For the metagenomic analysis, bar plots were plotted using ggplot2. Alpha diversity, the Shannon index and beta-diversity were calculated and results between groups compared using the non-parametric Kruskal Wallis test.

Results

Challenge with *C. parvum* provoked diarrhea and associated clinical signs in all but three calves. Colostrum supplementation did not significantly impact heart rate, respiratory rate or body temperature (P > 0.5). Calves in the *C. parvum*/MR group were more depressed (P = 0.01) on 6 dpc when compared with Sham group calves. Calves in the *C. parvum*/C group were not significantly different from Sham calves (P > 0.2). Calves in the *C. parvum*/MR group required significantly more OES treatments (P = 0.02) when compared with calves in the *C. parvum*/C group. Fecal shedding of *C. parvum* did not differ significantly between *C. parvum*/MR and *C. parvum*/C calves 4 dpc (57% and 14%, respectively; P = 0.27) or 6 dpc (86% and 57%, respectively; P = 0.56). Throughout the study, all calves were PCR negative for *E. coli* K99, *Salmonella spp*., and bovine rota- and coronavirus. Overall, *C. parvum* infection induced a drastic shift on the microbiome, partly contributed by *Clostridium spp*. overgrowth that was even more pronounced in *C. parvum*/MR calves. *C.parvum*/C calves displayed a microbiome profile more similar to the one observed in Sham calves than the *C. parvum*/MR group.

Conclusions

Colostrum supplementation showed limited effect in mitigating diarrhea but positively affected the associated depression and required treatments in calves challenged with *C. parvum*. Additionally, colostrum supplementation to *C. parvum* challenged calves appeared to modulate the fecal microbiota to a microbial composition more similar to that of unchallenged calves.

1471 - The dynamics of Mycoplasma bovirhinis-associated respiratory infections in dairy calves from Southern Brazil

Author: Selwyn Arlington Headley, Ana Paula Souza Frucch , Alais Maria Dall Agnol, Eloiza Teles Caldart, Rafaelli Ferreira Almeida, Ramiro Barros Madeira, Alice Fernandes Alfieri, Amauri Alcindo Alfieri

Objectives

Bovine respiratory disease (BRD) is a multifactorial and multietiological disease complex that is associated with a wide range of infectious disease agents. Additionally, abrupt alterations to management practices and environmental conditions are key elements for the developments of BRD. The exact participation of *Mycoplasma bovirhinis* in the development of BRD is controversial since this organism was identified in the lungs of cattle without any association with pulmonary disease. However, all studies that evaluated the possible association of *M. bovirhinis* in the development of BRD were done in North America, Europe, and Asia. This study investigated the respiratory infections dynamics associated with *M. bovirhinis* in calves from 15 dairy herds located in Southern Brazil.

Material and methods

Nasal swabs (NS) were obtained from asymptomatic (n=102) and calves with clinical manifestations (n=103) of bovine respiratory disease (BRD) and used in molecular assays to identify specific genes of viral and bacterial disease pathogens of BRD. These agents included *Histophilus somni, Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis* and mollicutes, *M. bovirhinis*, bovine viral diarrhea virus (BVDV), bovine alphaherpesvirus (BoAHV1), bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCoV), and bovine parainfluenza virus 3 (BPIV3), and ovine gammaherpesvirus 2 (OvGHV2).

Results

Only *M. bovirhinis*, BCoV, OvGHV2, *H. somni, P. multocida*, and *M. haemolytica* were detected in the NS of all calves during this study, with the most frequent infections associated with *M. bovirhinis*, BCoV, and OvGHV2. Infections due to M. bovirhinis were identified in diseased (57.84%; 59/102) and asymptomatic (55.33%; 57/103) calves at all farms. BCoV-related infections were diagnosed in diseased (51.96%; 53/102) and asymptomatic 51.43%; 53/103) calves. Similarly, infectious due to OvGHV2 occurred in diseased (37.25%; 38/102) and asymptomatic (27.18%/ 28/103) calves. Nevertheless, there was no statistical association for the occurrence of these pathogens when the two categories of calves were compared, except for OvGHV2-associated infections at few calves at one farm. Additionally, singular (n=55), dual (n=50), triple (n=41) , quadruple(n=25), and quintuple (n=9) infections were detected in diseased and asymptomatic calves. Furthermore, calves at most farms had comparatively more mixed infections (n=125) as compared to singular (n=55) infections. Singular infections at these farms were more frequent due to *M. bovirhinis* (45.45%; 25/55), followed by BCoV (25.45%; 14/55), *P. multocida* (10.9%; 6/55) and OvGHV2 (10.9%; 6/55). Infections due to *H. somni* were only diagnosed in two BRD calves from one farm. In addition, 12.19% (25/205) of all calves were not infected by any of the pathogens investigated. However, the nucleic acids of *M. bovis*, other mollicutes, BVDV, BoAHV1, BRSV, and BPIV3 were not detected in the NS from any of the calves during this study.

Conclusions

The results from this study probably represent the first investigation from Latin America to evaluate the possible association of *M. bovirhinis* in the development of BRD and provide data of the infections dynamics in Southern Brazil. Our results demonstrated that there was no significant statistical difference between infections due to M. bovirhinis in asymptomatic and diseased dairy calves, suggesting that this microorganism may not be associated with BRD, but a commensal of the respiratory tract of calves as weas previously indicated. The results from this study demonstrated that most calves (87.81%; 180/205) were either subclinically infected or developed clinical manifestations of BRD associated primarily with *M. bovirhinis*, BCoV, and OvGHV2. These results demonstrated that the respiratory infection dynamics of *M. bovirhinis* identified in calves from Southern Brazil is similar to that observed worldwide and suggest that there is not sufficient collected data to consider *M. bovirhinis* as a pathogen of respiratory infections in cattle. Additionally, the nonidentification of *M. bovis*, other mollicutes, BVDV, BoAHV1, BRSV, and BPIV3 from the NS of calves during this study suggest that these agents were not associated with the clinical respiratory manifestation observed in BRD calves.

1473 - Study of passive immunity against the Infectious Bovine Rhinotracheitis (IBR) transferred from dams previously immunised with gE-deleted marker vaccine to calves

Author: Stefano Petrini , Alessandra Martucciello, Cecilia Righi, Paola Gobbi, Giovanna Cappelli, Giulia Costantino, Silvia Pirani, Michela Pela, Carlo Grassi, Monica Giammarioli, Giulio Viola, Esterina De Carlo, Francesco Feliziani

Objectives

Bovine alphaherpesvirus 1 (BoAHV-1), belonging to the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae* under the family *Herpesviridae*, is an important pathogen of cattle. BoAHV-1 is responsible for several clinical forms, including Infectious Bovine Rhinotracheitis (IBR). To date, little information is available on the protective capacity of passive immunity (p.i.) transferred from dams previously immunized with IBR marker vaccines to calves (1). Therefore, in this study, we hypothesized that p.i. transferred from dams immunized with inactivated gE-deleted marker vaccine could protect calves from wild-type (wt) BoAHV-1. To test this hypothesis, we experimentally infected different groups of calves with p.i. to BoAHV-1 using a wt BoAHV-1.

Material and methods

Four groups of 3-6-month-old calves (A, B, C, D) were used for the experimental trial. Groups A and C had neutralizing antibodies (NAs) of 1.76 $\log_2$ and 0.75 $\log_2$, respectively, resulting from passive immunity. In contrast, groups B and D represent the negative controls. Subsequently, all animals were challenge infected with wt BoAHV-1 via the intranasal route at a dose of 5 x $10^{8.50}$ $TCID_{50}$/ml. Rectal temperatures were taken daily and clinical, virological (virus isolation) and serological investigations (gB-ELISA, gE-ELISA and virus-neutralization tests) were detected at different experimental times for 30 days.

Results

After challenge infection, all animals showed fever (> 40°C) 2-5 days post-infection (DPI) and typical clinical signs of IBR 2-10 GPI. In addition, all groups shed the virus 1-8 DPI with an average viral titre from 0.66 $TCID_{50}$/ml to 5.00 $TCID_{50}$/ml. Regarding the serological response obtained from groups A and C, these have exponentially increased their NAs titre until the end of the experiment, reaching titres ranging from 2.47 $\log_2$ to 2.97 $\log_2$. Groups B and D produced NAs after 15 DPI with an average titre of 2.50 $\log_2$ and 2.63 $\log_2$, respectively. These values increased until the end of the experiment, reaching titres of 3.10 $\log_2$ and 2.63 $\log_2$. Concerning the results in gB-ELISA, groups A and C showed positivity throughout the experimental period. In contrast, groups B and D seroconverted to gB after 15 DPI and maintained the same until the end of the experiment. Concerning the results obtained in gE-ELISA, all animals showed positivity after 15 DPI until the end of the experiments.

Conclusions

The results obtained in this study are partially in agreement with those published by other authors (2). In addition, they differ from those published by researchers, who have shown that p.i. protect the calves against experimental infection to BoAHV-1 (3-4). The findings of the present study demonstrated that p.i. evaluated here was not effective for the prevention of wt BoAHV-1 induced clinical disease. Therefore, the study's

hypothesis was not demonstrated. Further studies must be done to evaluate the IBR marker vaccines administered to calves with p.i. via intranasal and intramuscular routes.

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1475 - Use of an innovative non-endoscopic method for bronchoalveolar lavage in cattle under field conditions

Author: Ulrich Löschner, Marcus Klawitter, Thomas Breuer, Torsten Steppin, Ansgar Busch, Luis Leon

Objectives

In recent years, diagnostic samples of the respiratory tract are more frequently taken to rationalize antimicrobial use (Debruyne et al. 2022) and to define the pathogen causing Bovine Respiratory Disease Complex (BRDC). Different techniques like deep nasopharyngeal swabbing, bronchoalveolar lavage or transtracheal wash are commonly used (Van Driessche et al. 2017). This report details the use of an innovative **n**on-endoscopic method for **B**ronchoalveolar **L**avage (nBAL) in cattle under field conditions, that won the innovation prize at the WBC 2022. The aim of this report is to evaluate the on-field practicality, security, and efficiency of a novel nBAL system in collecting bronchoalveolar lavage fluid (BALf) from calves and to present some of the virological and bacteriological findings.

Material and methods

While providing routine veterinary support, six veterinarians from the tech service of Zoetis Germany used the nBAL examination set (Easy Lavage per Dr. Hasseler, QuIdee, Germany) to collect BALf's from calves with BRDC between January 2022 and December 2023. The used nBAL examination system consists of reusable, special curved and designed nasotracheal tubes with valve and insertion funnel and sterile consumables like single-use catheters, syringes, transport vessels and spikes for isotonic saline solution.

The BALf samples were pooled on farm level by maximal 5 calves and always analyzed by multiplex PCR and sometimes by an additional bacterial culture.

Results

Samples from 400 calves on over 80 commercial farms across Germany were collected. The nBAL is easily performed in a stable, no preparation or sedation of the animal was required, and only one assistant is required to briefly hold the animal. Compared with other methods, the collection of samples from calves takes only a few minutes even by non-expert users (Mean 2,9 min, max 4,35, min 1,4 min, n=10). The speed and minimal invasiveness of the nBAL method render the examination quite gentle on calves. Supervising veterinarians and farmers reported no complications without mortality on calves following the examination. The technique is easy to learn.

To obtain virological findings, only a multiplex PCR needs to be performed on the BALf samples. The samples collected from 79 calves from 20 farms show a distribution of pathogens as follows: BRSV-AG 20%; Pi-3-AG 0%, Bovine Coronavirus 33%, *M. bovis*-AG 43%, Pasteurella multocida-AG 90%, *Mannheimia haemolytica*-AG 33% and *Histophilus somni*-AG 40%.

Furthermore, the samples have a very low contamination level (reports of the laboratory findings, non-significant commensal overgrowth) which is conducive to obtaining microbiologically mono-bacterial results leading to a clinically interpretable culture result. In the described BALf samples above, bacterial growth was found 64 times with a distribution of pathogens as follows: *Pasteurella multocida* 55%, *Mannheimia*

haemolytica 33%, *Trueperella pyogenes* 6%, *Histophilus somni* 5%, *Staphylococcus aureus* 2%.

For greater sustainability the nasotracheal tubes can be reused and are easy to clean and disinfect.

Conclusions

This report agrees with Dr. Hasseler experiences on the new nBAL system being a cost-effective facilitating clinical interpretation and bringing in a higher return on investment in bacteriologic culturing and minimal invasive method for extracting BALf from calf lungs. The method allows to sample many calves in a short period of time. The sample quality offers specific pathogen diagnostics for BRDC. We recommend the nBAL method for daily veterinary practice for targeted supportive therapy and prophylactic decisions to the BRDC.

Reference

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Van Driessche et al (2017): A deep nasopharyngeal swab versus nonendoscopic bronchoalveolar lavage for isolation of bacterial pathogens from preweaned calves with respiratory disease. J Vet Intern Med 2017;31: 946–953

1481 - Assessing the impact of clavulanic acid and prednisolone on common mastitis-causing bacteria: An in-vitro-study on antibiotic efficacy

Author: Franziska Preine, Nicole Wente, Yanchao Zhang, Luis Leon, Volker Krömker

Objectives

Antibiotics play a crucial role in inhibiting or killing microorganisms, such as bacteria, and are essential in treating infections. However, there are some mastitis bacteria that are able to render ß-lactam antibiotics ineffective, e.g. by synthesizing enzymes such as ß-lactamases. Clavulanic acid, which is often combined with antibiotics such as amoxicillin, acts as a ß-lactamase inhibitor, which prevents the bacterial inactivation of antibiotics and thus extends their spectrum of action. Despite structural similarities to ß-lactam antibiotics, clavulanic acid lacks significant antibacterial effects. Instead, its ß-lactam ring competitively inhibits bacterial ß-lactamase, enabling antibiotics to exert their effects. In veterinary medicine, a common combination is amoxicillin with clavulanic acid and prednisolone [like Synulox® LC Plus, Zoetis Germany], which is widely used for the treatment of bovine clinical mastitis. Prednisolone is a drug from the group of glucocorticoids with an anti-inflammatory effect. It shows immunomodulation in the bovine udder gland (1) and in bovine cells (2). It is discussed whether clavulanic acid or also Prednisolone alone has an antibiotic effect on mastitis pathogens and whether the product must therefore be considered a combination product. Our study investigates the antibiotic efficacy of clavulanic acid and prednisolone against common mastitis-causing pathogens *in-vitro.*

Material and methods

The efficacy of clavulanic acid against common mastitis pathogens was determined on the basis of the minimum inhibitory concentration. The pathogen isolates came from the strain collection of the Hanover University of Applied Sciences and Arts and were obtained from clinical mastitis cases in German dairy cows. The total of 20 isolates examined can be divided into Escherichia (E.) coli (5), Staphylococcus (S.) aureus (5), Streptococcus uberis (5) and non-aureus staphylococci (NAS) (5). In addition, a ß-lactamase-positive S. aureus isolate was examined. E. coli ATCC 25922, Enterococcus faecalis ATCC 29213, Klebsiella pneumoniae ATCC 700603, and Staphylococcus aureus ATCC 29213 were used as reference strains. The sensitivity of these pathogens to the individual active substances clavulanic acid (0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L, 1000 mg/L), prednisolone (2.5mg/L) and amoxicillin (50mg/L) was tested. In addition, the sensitivities to a combination of amoxicillin/clavulanic acid and amoxicillin/clavulanic acid/prednisolone were determined.

The sensitivity test was carried out using the microdilution method. Sterile microtiter plates were filled with different drug concentrations in Mueller-Hinton broth at 100µl/well each. Subsequently, 5µl microorganism suspension with a concentration of 10^7 cfu/mL was added to each well. The inoculated microtiter plates were incubated at 36°C for 24 hours. Turbidity of the respective microplate well after the incubation period was characterized as bacterial growth, which means that the microorganisms are not sensitive to the active ingredient concentration.

Results

All pathogen isolates examined showed growth in the wells of the microtiter plates coated with all of the different concentrations of clavulanic acid after 24 hours. Thus, none of the pathogens showed sensitivity to this active substance. The same result was seen with prednisolone 2.5 mg/mL. In contrast, most pathogens (19 out of 20, only one E. coli showed resistance) were sensitive to drug combinations amoxicillin/clavulanic acid (8/4 mg/L) and amoxicillin/clavulanic acid/prednisolone (50/12.5/2.5 mg/L).

Conclusions

Results indicate that clavulanic acid alone has no antibiotic activity, particularly against typical mastitis-causing Gram-positive and Gram-negative bacteria. Combined with amoxicillin, its efficacy is evident, ensuring a broader spectrum against resistant strains. Given recent German regulations (3), veterinarians must report antibiotic use in dairy cows. This study underscores that clavulanic acid alone does not exhibit antibiotic properties against mastitis pathogens, emphasizing its role as an adjunctive therapy rather than a standalone antibiotic.

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1482 - Survey on the use of on-farm diagnostic for clinical mastitis among veterinarians in Germany

Author: Ansgar Busch, Luis Leon, Ulrich Löschner, Thomas Breuer, Torsten Steppin, Marcus Klawitter

Objectives

Treatment of clinical mastitis in cattle with antibiotics is widespread. In Europe, guidelines for the prudent use of antimicrobials in veterinary medicine encourage the use of rapid diagnostic tests/on-farm culture (OFC) to identify pathogens causing mastitis and to minimize the use of intramammary and injectable antimicrobials in dairy cows (European Commission Notice 2015/C 299/04).

The use of OFC before treatment shows economic benefits for farmers (30 EUR cost reduction per case) and a reduction in antibiotic use by up to 60% (Schmenger et al. 2022). OFC has no negative impact on healing, somatic cell count, milk yield, recurrence of mastitis or culling (De Jong et al. 2023).

Since successful implantation of OFC requires changes in udder health management, it is key to understand the mindset, perspective and understanding of German bovine veterinarians. In addition, responses to the new test VetScan Mastigram+® (Zoetis Inc. USA) a novel lateral flow test that detects Gram-positive-bacteria in the milk after 7 to 7.5 hours incubation by 37°C, were analyzed.

Material and methods

The study was conducted in Germany to report the attitudes of bovine veterinarians regarding the use of OFC to treat clinical mastitis. The veterinarians were personally visited to respond to a questionnaire about the use of OFC in mastitis, briefly explain Mastigram+® and discuss different OFC methods.

Results

63 cattle veterinarians were surveyed from September to December 2023 in various German regions, predominantly in Bavaria (62%). The clinics perform udder health diagnostic in external labs (86%), with OFC (35%), in their own clinical lab (32%) or do no diagnostic (7%) (multiple choice). The interviews showed that 57% of the vets do not use OFC; the other 43% used OFC either in the clinic (28%) or on the farm (11%) (multiple choice). Veterinarians mainly (44%) use the VetoRapid® test (Vetoquinol, France), 33% use MastDecide® (QuIdee, Germany) and 27% use Speed Mam color® (Virbac, France). 90% of the farmers (90%) use MastDecide® (QuIdee, Germany).

Most veterinarians agreed that the new lateral flow test offers clear advantages: 83% described the test as very fast, 58% said it is easy for farmers to use and interpret and 58% said it contributes to reduce antibiotics in livestock according to the new German and European legislations. 83% of veterinarians would implement the use on dairy farms and 33% in their own clinic and in dairies. 83% of the veterinarians would implement the use on farms that need to reduce antibiotics (50%) and on farms with udder health problems (42%).

Conclusions

In the area of clinical mastitis treatment, increased diagnostic efforts will be necessary in the future to be able to use antibiotics in a more targeted manner. One possible option is to use rapid on-farm tests, which can often avoid unnecessary use of antibiotics.

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1493 - To treat or not to treat - Understanding the antibiotic use in clinical mastitis among German dairy farmers and the role of on-farm culture as a tool for antibiotic reduction.

Author: Marcus Klawitter, Luis Leon, Ansgar Busch, Ulrich Löschner, Torsten Steppin, Maria Elena Vergara Hernandez

Objectives

The growing number of antibiotic-resistant bacteria poses a problem for human and animal health. The European Commission will reduce the sales of antimicrobials for farmed animals and in aquaculture by 50% in 2030 (European Commission; 2020).
Many of the antibiotics in dairy cow farming are used to treat mastitis. The use of On-Farm Culture (OFC) before clinical mastitis treatment can be a tool for a prudent use of antibiotics in the dairy industry. OFC has no negative impact on healing, somatic cell count, milk yield, recurrence of mastitis or culling (Bochert and Heuwieser 2022; de Jong et al. 2023). Since successful implementation of OFC requires a change in farmers' mindsets, the present study was conducted to survey the behaviors, expectations and needs of German dairy farmers.

Material and methods

From September to December 2023, a survey of German dairy farmers was conducted about their attitudes and the future relevance of antibiotic reduction and the use of OFC as a decision-making tool for the treatment of clinical mastitis.
30 dairy farmers (mean 211 cows/farm, min 22 cows/farm, max 1.200 cows/farm; 13,3% organic farms) from Northwest Germany were randomly selected and personally contacted and invited to participate in the survey responding to a structured questionnaire. The selected region is a large livestock producing area with high antibiotic consumption (German Federal Office of Consumer Protection and Food Safety, BVL, 2023). The questionnaire is similar to the study by Preine et al. (2022), in which 97 dairy farmers in different regions of Germany were surveyed between March and October 2021.

Results

6,6% of farmers responded that all cases of mastitis must be treated immediately with antibiotics. The results are very similar to those of Preine et al. (7,2%). Many farmers (90%) answered that antibiotics would have to be used more specifically in the future (Preine et al. 96.9%). In future, farmers will need to use OFC for mastitis (56,6% of responses). 16,6% of farmers use OFC in their farms, and the study shows that 70% of non-users are interested in OFC but 20 % think that their veterinarians are not interested in OFC (Preine et al. 16,5%). The main reasons for the dairy farmers for non-use are currently “no interest/no need” (55%) and “too much overtime” (40%).

Conclusions

The study confirmed previous studies and shows that a large proportion of farmers are interested and willing to use more on-farm diagnostics in the future and thus treat clinical mastitis more selectively. Farmers realize that they need to change management and that they have time to decide before treatment. This willingness should be used to establish selective therapy concepts based on immediate on-farm diagnostics in the future.

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1516 - IDV surveillance in the North Italian cattle population (Emilia-Romagna and Lombardy Regions) between 2018-2023

Author: Alice PROSPERI , Ana Moreno, Laura Baioni, Francesca Palumbo, Silvia Faccini, Carlo Rosignoli, Camilla Torreggiani, Giovanni Pupillo, Chiara Anna Garbarino, Laura Soliani, Giovanni Alborali, Andrea Luppi, Chantal Snoeck, Chiara Anna Chiapponi

Objectives
Influenza D virus (IDV), firstly identified in 2011, has been detected in a wide host range of animal species and cattle are considered the major viral reservoir. The virus efficiently replicates and transmits in ferrets, which are the chosen animal model for Influenza A virus transmission to humans.
The IDV genus was originally divided into 3 main genetic and antigenic clusters based on the Hemagglutinin-esterase (HEF) gene phylogeny, which were D/OK (worldwide distributed), D/660 (circulating in the USA), and D/Japan (in Japan), even if this nomenclature is no longer adequate considering the viral diversity.
Since 2014 the co-circulation of both D/OK and D/660 has been reported in the USA, and reassortment events within the two viral lineages have been highlighted.
In this study, IDV circulation in Northern Italy and the viral genetic and antigenic diversity was investigated.
Material and methods
Between January 2018 and November 2023, 2713 samples (1684 nasal swabs, 905 lung tissues and 124 bronchoalvear fluids-BAL), collected during Bovine Respiratory Disease Complex (BRDC) outbreaks in Emilia-Romagna and Lombardy Regions (North Italy), were investigated for IDV presence using a real time RT-PCR previously described (Faccini et al. 2017). Viral isolation was attempted on real time RT-PCR positive samples. Viral isolates or PCR positive clinical samples were sequenced by Next Generation Sequencing using Illumina technology and phylogenetic analyses were performed.
Results
Seven lung tissues, 12 BAL and 148 nasal swabs were IDV positive (total 167 samples), and full genome sequencing was performed from 109 samples. Considering the HEF gene, 72 of them were identified within the previously reported European lineage (D/OK) and 37 strains clustered with the American D/660 lineage, which was firstly reported in Europe in 2018 in Italy (Chiapponi et al. 2019).
Moreover, since 2019 reassortant viral strains were detected and, after a period 2019-2022 with higher prevalence of D/660 strains, we found an increased circulation of D/OK in 2023.
Conclusions
IDV risks for animal and human health and its role in the BRDC is not clearly identified yet, but its frequent detection during BRDC metagenomics studies may suggest a potential role as a door opener emerging pathogen, thus further studies on its ecology and epidemiology are certainly needed.
In March 2018, D/660 lineage was firstly reported in Europe in Italy, but D/660 strains are now circulating in France, Denmark and the Netherlands. Our data suggest the rapid spread of D/660 strains, co-circulating with D/OK in the bovine population, with evidence of reassortant events, as already occurred in USA. According to our surveillance, in Northern Italy most of the IDV strains in 2018 belonged to the D/OK

lineage, meanwhile between 2019-2022 an increasing of D/660 strains was observed, but in 2023 D/OK strains were the most prevalent. In this five-years surveillance the co-circulation of both clades was observed, sometimes identified in a single farm, which promoted the emergence of reassortant strains.
These data update the IDV genetic and antigenic diversity surveillance in Europe.

Acknowledgements:
This research was partially supported by

- EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) and by Italian Ministry of Health, grant numbers RC IZ LER 2018009 and RC IZ LER 2020005.
- ICRAD Preventer: Deciphering the role of influenza D virus in bovine and human respiratory diseases in Europe. EFSA partnering grant GP/EFSA/ENCO/2020/03: Developing an integrated approach to assess the emergence threat associated with influenza D viruses' circulating in Europe.

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1518 - Cattle botulism investigations in the Emilia-Romagna (Italy) risk-area: an update

Author: Alice PROSPERI , Camilla Torreggiani, Chiara Anna Garbarino, Roberta Taddei, Marco Tamba, Carlo Rosignoli, Concetta Scalfaro, Fabrizio Anniballi, Luca Bano, Cosimo Paladini, Chiara Chiapponi, Andrea Luppi

Objectives

Botulism is a human and animals' sporadic disease caused by botulinum neurotoxins (BoNTs), which are thermolabile toxins produced by bacteria belonging to the genus *Clostridium*. Recently, genes homologous to the ones encoding BoNTs were detected also in different microbial species' genome (*W. oryzae*, *C. piperi*, *E. fecium*).

Analyzing the bovine botulism outbreaks identified in the Emilia-Romagna region (Italy) in the period 2014-2023, it is possible to highlight how of the 28 outbreaks, 27 involved the provinces of Parma and Piacenza, and were in a well-defined area bordering the rivers Ongina and Arda. The outbreaks increase in recent years and the high incidence in the risk area represent an exceptional and alarming problem, which requires further epidemiological investigations. Furthermore, the risk area identification has led the Official Veterinary Service to the authorization of autogenous or commercial vaccines.

Material and methods

The bovine botulism outbreaks between 2014 and 2023 were investigated using the National Reference Center for Botulism (CNRB-ISS) methods: culture method, mouse test (ISS N-RL CNRB30.012 rev 2 2021 and prior) and multiplex real-time PCR (ISS N-RL CNRB31.012 rev 2 2021 and prior). The outbreak diagnosis was carried out as far as possible using an association of analytical and biomolecular methods, in compliance with 2010/63/UE. The analyzed samples were collected from symptomatic/deceased animals or from the environment.

The outbreaks trend in the period was evaluated using the data about the botulism diagnosticated outbreaks garnered from IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna) archives and the mortality within the individual outbreak was gathered from the BDN (Banca Dati Nazionale) (https://www.vetinfo.it/).

To obtain a risk assessment in the outbreaks, a checklist specific for the cattle botulism risk analysis and biosecurity was carried out (data collection still ongoing).

Results

The botulism diagnosis was confirmed in 26 out of 28 cases and in most of these the presence of the DC toxinotype was confirmed. Type D was identified in two outbreaks in 2014, however the test method used at that time did not allow the distinction of chimeric variants DC. In 27 cases out of 28, the outbreaks were in the risk area within Parma-Piacenza provinces.

The checklist was designed involving several experts and the CNRB-ISS; this contains the analysis of farming and environmental parameters such as the evaluation of the farm internal/external biosecurity. The checklist will be submitted retrospectively in the botulism outbreaks within the risk area (still ongoing).

Conclusions

In cattle farms, botulism causes huge economic losses and is most frequently caused by the assumption of feed contaminated with BoNT-producing bacteria spores (foodborne form).

This study aimed to investigate the trend of bovine botulism episodes in the period 2014-2023 in the Emilia-Romagna region. The data has shown outbreaks increase in recent years and a high incidence of cases in the provinces of Parma-Piacenza. Further epidemiological investigations are necessary to identify shared risk factors, which are essential to contrast and control the health problem described.

Acknowledgements:
This research was partially supported by IZSLER funding within the AUTOFIN_BOTULBOV – BOTULISMO BOVINO: INDAGINE EPIDEMIOLOGICA DEI FOCOLAI NELL'AREA DI RISCHIO DELLE PROVINCE DI PARMA E PIACENZA.

1525 - Antimicrobial Use in the Prophylaxis (Prevention) and Metaphylaxis (Control) of Calf Diarrhea: A Scoping Review

Author: Christian Bernal-Córdoba , Rúbia Branco-Lopes, Caroline Minjee-Lee, Diana Pérez-Solano, Erik Fausak, Noelia Silva-del-Río

Objectives

This scoping review (**ScR**) aimed (1) to identify and describe the scientific literature on the use of antimicrobial drugs (**AMDs**) for calf diarrhea prophylaxis and metaphylaxis, (2) to characterize the assessment methods employed for evaluating calf health and disease, and (3) to evaluate the feasibility of conducting meta-analyses (MAs) to assess the efficacy of AMDs for calf diarrhea prophylaxis and metaphylaxis.

Material and methods

This ScR adhered to a pre-established protocol available on the University of California, Davis eScholarship (https://escholarship.org/uc/item/0nw528h4). The literature search was conducted in July 2019, utilizing four electronic databases (CAB Abstracts, Medline, Scopus, and Biosis). A backward and forward citation (manual) search was conducted to capture trials possibly overlooked in conventional databases. Subsequent search updates occurred in June 2020, February 2022, and May 2023. Eligibility criteria encompassed controlled trials employing prophylactic and metaphylactic AMD interventions for diarrhea in calves aged ≤6 months. Trials evaluating AMD efficacy based on health outcomes [e.g., fecal consistency (**FC**), dehydration], fecal pathogen shedding (e.g., oocyst excretion), or a combination thereof were included. To enhance reliability and mitigate bias, the screening and data extraction processes underwent independent evaluation by two reviewers with backgrounds in veterinary medicine and expertise in research synthesis methods.

Results

Search results: The database search yielded 3,214 references, with additional 46 references from the manual search, resulting in 3,260 identified references for screening. After screening, 42 manuscripts encompassing 62 individual trials were included in the ScR. **Trial characteristics:** The majority of trials were conducted in Europe (48.4%) and North America (43.5%) and were published between 2000 and 2021 (51.6%). Trial sample sizes ranged from 8 to 513 calves (mean = 60±79). Only two (3.2%) trials reported sample size calculations (POWER procedure). Most trials (74.6%) exhibited incomplete or missing reporting on randomization methods. Approximately two-thirds (61.3%) of the included trials were industry-funded or had industry authorship. Reporting on the blinding status of treaters (22%), observers (24%), and data analysts (0%) was infrequent among trials. **Etiological agent:** Causative agents identified in the included trials were primarily *Eimeria* spp. (41.9%), followed by *Cryptosporidium parvum* (40.3%) and *Salmonella typhimurium* (8.1%). **Outcomes:** In the included trials, FC (93.5%) and stool blood (48.4%) were the most frequently evaluated fecal traits, alongside common health outcomes such as appetite (27.4%) and attitude (25.8%). There was notable heterogeneity in health outcome assessment methods, often with a lack of comprehensive reporting. In terms of laboratory outcomes, optical microscopy was the most frequently employed technique for assessing fecal oocyst shedding of *Eimeria* spp. (41.9%) and *C. parvum* (33.9%). Bacterial culture predominated in identifying *S. typhimurium* (12.9%), while immunodiagnostic tests were the primary

method for detecting *Escherichia coli* (8.1%). **Disease definition:** Despite all trials evaluating diarrhea, only 22.6% (n = 14) of these disclosed a definition of diarrhea. Among these trials, the definition of diarrhea was based on a single metric (FC; 92.9%; n = 13/14) or two metrics (FC + fecal dry matter; 7.1%; n = 1/14). **MAs:** The ScR results suggest the potential for conducting MAs to assess AMD efficacy in diarrhea caused by *C. parvum* and *Eimeria* spp. For *Eimeria* trials, a sufficient number exists for diclazuril (n = 9), toltrazuril (n = 7), lasalocid (n = 6), and decoquinate (n =4), indicating the feasibility of MAs for these AMDs. Similarly, enough trials assessed halofuginone (n = 16) for calf cryptosporidiosis, suggesting the feasibility of pursuing MAs for this AMD. Conducting MAs to assess AMD efficacy in diarrhea caused by *Salmonella* spp. and *E. coli* is not feasible due to the scarcity of trials and high variability in AMD types and treatment specifics.

Conclusions

For future trials evaluating the efficacy of AMD in calf diarrhea, adherence to regulatory authorities' treatment specifications and compliance with established reporting guidelines, such as the REFLECT (Reporting Guidelines for Randomized Controlled Trials in Livestock and Food Safety) statement, is imperative. Addressing these aspects will enhance the transparency and comparability of trial outcomes. Furthermore, there is a critical need for additional research to standardize a definition of diarrhea and validate methods for assessing health outcomes. Establishing a consensus in these areas will contribute significantly to the precision and reliability of calf disease and health evaluations.

1531 - Bovine preputial epithelium produces antibodies against Tritrichomonas foetus following aerosol application of synthetic mRNA

Author: Merrilee Thoresen, Elmer Heath King, Santiago Cornejo, Cassie Barber, Daryll Vanover, Hannah Peck, Jae Yeon Joo, Jean Feugang, Philip John Santangelo, Amelia Woolums

Objectives
The current study evaluated levels of *Tritichomonas foetus* (Tf) specific bovine IgG in preputial skin of bull calves following aerosol treatment with synthetic mRNA encoding for antibody against cell surface antigen Tf 1.17. Our goal was to determine if there were differences in expressed levels of antibodies three weeks following treatment, when using a low or high dose of synthetic mRNA for expression of either a secreted or membrane anchored version of the antibody.
Material and methods
Four treatment groups consisting of 3 bull calves each included a high (3 mg) or low (1.5 mg) dose of synthetic mRNA, encoding for expression of either a secreted or membrane anchored version of antibodies against Tf. Additionally, two negative control calves received no mRNA treatment. The light chain sequence included a NanoLuc reporter label to facilitate identification of the expressed protein and differentiate it from naïve bovine IgG. Synthetic mRNA was suspended in molecular grade water (carrier) and applied via a mucosal atomization device into the preputial fornix of each calf. Negative control calves were treated with carrier only. Three weeks following treatment, calves were euthanized, and preputial epithelium was collected and frozen. Diluted NanoGlo (Promega) substrate was applied to thawed skin samples and they were imaged with an in vivo imaging system (IVIS). Areas exhibiting high radiance were excised via an 8 mm punch biopsy. Protein lysates were prepared from the biopsies and capillary based western blots for NanoLuc labelled bovine IgG antibody were prepared and imaged via a Jess automated system (biotechne).
Results
Skin samples from calves that were treated with the high dose (3 mg) of secreted or anchored mRNA constructs had areas of radiance that ranged from 1.8-2.4 x 10^5, while low dose calves exhibited no radiance following background correction when imaged with IVIS. In contrast, NanoLuc labelled bovine IgG was detected in western blots of skin lysates from all the calves that were treated with synthetic mRNA. Chemiluminescent signals were detected, and the bands were consistent with products the size of bovine IgG with NanoLuc labelled light chains.
Conclusions
Detection of NanoLuc in the skin samples from treated calves, via both IVIS imaging and western blot, confirmed that aerosol application of synthetic mRNA to preputial skin induced the production of bovine IgG against the Tf 1.17 cell surface antigen. Using synthetic mRNA to produce antibodies against Tf at the site of infection, in combination with systemic vaccination, could provide a new therapeutic approach to prevent or clear Tf infection in bulls.

1549 - Ovine gammaherpesvirus 2 infections in water buffaloes (Bubalus bubalis) from Central western, Brazil

Author: Selwyn Arlington Headley, Juliana Torres Tomazi Fritzen, Natalia Zaparoli Zucoloto, Mariana Motta Castro, Ana Clara Rodrigues Stella, Vanessa Resende Rocha Tavares, Rafaelli Ferreira Almeida, Caio Cabrera Antunes Santos, Allana de Andrade Mathias, Ana Teresa Alves Chiaratto, Ana Flávia Ferreira Domingues, Bruna Suntack Adorno, Thiago Monteiro Lopes, Ramiro Barros Madeira, Amauri Alcindo Alfieri

Objectives

Introduction

Ovine gammaherpesvirus 2 (OvGHV2), is a member of the genus *Macavirus*, subfamily *Gammaherpesvirinae* and is the cause of sheep associated-malignant catarrhal fever (SA-MCF) in which sheep are the asymptomatic carrier-hosts with infections and/or clinical diseases occurring in a wide range of susceptible mammalian populations. SA-MCF occurs worldwide and is also endemic in Brazil with infections occurring principally in cattle. Alternatively, there are comparatively few confirmed descriptions of infections by OvGHV2 in water buffaloes (*Bubalus bubalis*) worldwide. Furthermore, OvGHV2 can produce subclinical infections as well as the classical clinical syndromes of SA-MCF in affected ruminants. This study describes the molecular detection of OvGHV2 infections in water buffalos from Central western, Brazil.

Material and methods

All buffaloes from this study originated from the same farm located in the State of Goiás, Central western, Brazil. Sheep are not reared concomitantly with buffaloes at this farm, but there are herds of sheep within proximity. Manifestations of clinical disease were not identified nor reported. Tissues were collected from all buffaloes during slaughter and routinely processed for molecular analyses. Pulmonary, renal, and intestinal fragments from 37 water buffaloes were received, and maintained at -80°C until used in molecular assays. Nucleic acid extraction was done using a combination of the phenol/chloroform/isoamilic acid and silicon/guanidine isothiocyanate techniques. The extracted DNA was then used in molecular assays designed to amplify the 238 base pair (bp) fragment of the OvGHV2 tegument protein gene using the Baxter primers. The obtained amplicons were purified (Wizard SV Gel and PCR Clean-Up System; Promega Corporation), quantified (Qubit Fluorometer; Invitrogen Life Technologies, USA), and sequenced using an ABI3500 Genetic Analyzer sequencer with a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The obtained nucleotide sequences were compared with those deposited in GenBank.

Results

The Baxter primers amplified the desired bp of the OvGHV2 tegument protein gene in 18.9% (7/37) of all buffaloes evaluated. Direct sequencing confirmed the nested-PCR assay. The nucleotide sequences derived from all buffaloes had 100% sequence identity each other and with the reference strain of OvGHV2, and between 98-100% nucleotide sequence homology with other OvGHV2 strains from diverse geographical regions. Infections by OvGHV2 were identified more frequently in the lungs (85.7%; 6/7), followed by the kidneys (71.4%; 5/7), and small intestine (57.1%; 4/7) of these buffaloes. Additionally, OvGHV2 DNA was identified within all organs evaluated from three

buffaloes; while infections occurred only in the lungs of three buffaloes, the kidneys of two, and in the small intestine of one buffalo.

Conclusions

These findings demonstrated that 18.9% (7/37) of the asymptomatic buffaloes were infected by OvGHV2, with infections being widespread in all organs evaluated of three buffaloes. Consequently, these buffaloes were subclinically infected by OvGHV2; similar subclinical infections associated with OvGHV2 were previously described in the American bison and cattle. As far as the authors are knowledgeable, the results of this investigation represent the second study to diagnose OvGHV2 in buffaloes from Brazil. In the first investigation, an outbreak of clinical SA-MCF occurred in Murrah buffaloes that were reared concomitantly with sheep and cattle at the same farm. Alternatively, in the present study, there was no comingling between the infected buffaloes and the asymptomatic carrier-hosts (sheep) for OvGHV2, while all buffaloes were subclinically infected. The detection of OvGHV2 in ruminants reared without any direct contact with sheep was also identified in previous outbreaks of SA-MCF and in subclinical infections of OvGHV2 in cattle from diverse geographical regions of Brazil. These findings may suggest that there may be another carrier-host for OvGHV2 in this country. Additionally, the frequent diagnosis of subclinical OvGHV2 infections in ruminants from Brazil may suggest that the real prevalence of OvGHV2 in this continental nation is more elevated than previously described. Accordingly, a seroepidemiological survey is being developed to determine the prevalence of OvGHV2 in selected ruminant populations from Brazil.

1554 - Attenuation evaluation of two novel live vaccine candidates against bovine group A rotavirus

Author: Dong-Jun An , Bang-Hun Hyun, Gyu-Nam Park, SeEun Choe

Objectives

Morbidity and mortality rates due to bovine group A rotavirus (BoRVA) infections are high, resulting in direct and indirect economic losses to the beef and dairy industries. In general, BoRVAs infect calves aged less than one month, particularly calves younger than 10 days of age. BoRVA G8P[7] was the predominant type of Korean calve infection in the past, but a novel G6P[5] emerged in Korean calves around 2014. Currently, the G6P[5] genotype is the main causative agent in Korean calf infections, followed by G8P[5]. Therefore, we isolated the G6P[5] genotype (17A4-12 strain) and the G8P[5] genotype (17A21-2 strain) to develop two new vaccines from the recently prevalent strains.

Material and methods

To create an attenuated virus using TF-104 cells, the isolated two strains (17A4-12 and 17A21-2) were continuously passaged up to 120 times (p120) and 100 times (p100), respectively. The virulence of the two attenuated BoRVA strains was inoculated orally twice in 7-day-old newborn calves (non-colostrum-fed, negative for BoRVA antigen and antibody), and clinical symptoms were observed for 14 days.

Results

During the two weeks, calves inoculated with the attenuated BoRVA strains showed no specific clinical symptoms (vomiting, acute diarrhea, lethargy, and loss of appetite). Additionally, viral shedding through feces and the presence of viral antigens in intestinal organs were not detected. Histopathological examination of the calf's intestines (duodenum, jejunum, and ileum) showed no significant changes (villous atrophy, epithelial cell necrosis, vacuolation, etc.) due to viral infection.

Conclusions

In conclusion, two live attenuated vaccine candidates (17A4-12-p120 and 17A21-2-p100) were finally selected according to the recently prevalent BoRVA genotypes in South Korea. In the future, we plan to conduct tests to determine whether attenuated candidate strains selected from calves will return to pathogenicity.

1555 - Characterization of a live attenuated bovine coronavirus vaccine KBR-1 strain

Author: Gyu-Nam Park, Bang-Hun Hyun, Dong-Jun An , SeEun Choe

Objectives
Bovine coronavirus, the main cause of diarrhea in calves, results in high economic losses for cow farmers. In South Korea, calf diarrhea, which affects newborn calves, and winter dysentery, which occurs in adult cows during the winter, are detected continuously. The vaccine strain (BC94) used in South Korea belongs to GI type; however, phylogenetic analysis revealed that all of the prevalent circulating strains belong to the GIIa type. Therefore, we attempted to develop a live attenuated BCoV vaccine candidate that targets recent prevalent strains.
Material and methods
The bovine coronavirus (BCoV) KBR-1 strain was obtained from calf diarrhea samples in 2017 and belongs to the GIIa group. To attenuate this strain, it was subcultured continuously in HRT-18 cells up to 79 times, followed by 80–120 passages in MDBK cells. Seven-day-old calves (negative for BCoV antigen and antibodies) that did not consume colostrum were orally inoculated with the attenuated candidate strain (KBR-1-p120), or with KBR-1 passaged 10 times (KBR-1-p10) in HRT-18 cells.
Results
The KBR-1-p120 strain harvested from MDBK cells at passage 120 harbored 13 amino acid mutations in the spike gene. Additionally, the KBR-1-p120 strain showed high viral titer and cytopathogenic effects in MDBK cells. Calves inoculated with KBR-1-p10 had a low diarrhea score, and BCoV RNA was detected at 3–7 days post-inoculation (DPI). The virus was also present in the duodenum, jejunum, and ileum at autopsy. However, calves inoculated with KBR-1-p120 had low levels of BCoV RNA from fecal at 4-6 DPI, no diarrhea, and an extremely small amount of BCoV RNA was present in the jejunum and ileum at autopsy. The small intestines of calves inoculated with KBR-1-p120 were emulsified and used to infect calves two more times, but recovery of pathogenicity did not occur.
Conclusions
Therefore, the KBR-1-p120 strain has the potential as a live vaccine candidate.

1561 - Detection of Coxiella burnetii in fetal thymus and placenta of cattle in Brazil

Author: Maria Angeles Ramo Gil, Lilian Gregory , Lucas Alencar Fernandes Beserra, Gisela Gregoria Choque, Marcia Mayumi Fusuma, Liria Hiromi Okuda

Objectives
Due to the negative impacts of coxiellosis on cattle farming, risks to public health and lack of data on the disease situation in Brazil, this work aims to describe the detection of Coxiella burnetii in abortions of bovine females in Brazil.
Material and methods
In this study, thirteen thymuses and fifteen placentas were used. As inclusion criteria, negative aliquots were used for brucellosis, toxoplasmosis, bovine viral diarrhea, neosporosis and infectious rhinotracheitis. The research was carried out at the Bovine Virus Laboratory of the Biological Institute of the State of São Paulo, SP, Brazil. Molecular diagnosis was carried out using the quantitative PCR technique using the EXOone Coxiella burnetii® commercial kit. DNA extraction from the samples was carried out using the commercial MagMAXTM CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific Inc., Waltham, MA, United States) and amplification of the pathogen was carried out using quantitative PCR, using the EXOone Coxiella burnetii commercial kit. ® (EXOPOL S.L.U) following the manufacturer's recommendations.
Results
The agent was detected in 23.1% (3/13) of fetal thymus samples and 13.3% (2/15) of placentas. In Brazil, this is the first study that describes the presence of the agent in the placenta.
Conclusions
There is no description of the fetal thymus in the world literature. In the country, the impact of these findings on cattle farming is still scarce, however there are some reports in the literature of the pathogen's circulation on Brazilian rural properties. Given the negative effects of coxiellosis on livestock and the risks to public health, it is necessary to implement surveillance measures, and this agent must be included in the list of differential diagnosis of cattle abortion in Brazil, implementing diagnostic and control measures, as well such as, actions in single health in the face of occupational exposure in livestock farming.

1565 - Prevalence of ESBL-producing bacteria in neonatal dairy calves in different regions of Northern Italy, preliminary results

Author: Laura Filippone Pavesi, Martina Penati, Laura Musa, Camilla Fumagalli, Guido Grilli, Maria Filippa Addis, Valerio Bronzo

Objectives

This study aimed to determine the presence and prevalence of extended-spectrum β-lactamases (ESBL)-producing bacteria in feces from dairy calves located in dairy farms on three different regions of Northern Italy.

Material and methods

The farms were included in the study according to the score of the Biosecurity Checklist used by public health veterinarian and the presence of at least one case of neonatal calf diarrhea during the last year. For each farm, healthy and diarrhoeic calves, aged between 1 and 21 days of life, were enrolled. After identifying the calves, a clinical examination was performed, and the vitality, fecal, and dehydration scores were recorded.

Upon approval of the Welfare Ethical Committee of the University of Milan, according to the herd size, a variable number of calves per farm was fecal sampled. All samples were stored at 5 °C and analyzed within 24 hours, using the following protocol.

0,1g of fresh feces were added to Mueller Hinton broth and the tubes were incubated for 24h at 37°C. Then, 100 µL of each culture was plated on MacConkey and CHROMagar™ ESBL and incubated for 24 h at 37°C in aerobic condition. Using the direct transfer method, the species identification was accomplished via matrix-assisted laser desorption-ionization mass spectrometry (MALDI-TOF; Bruker Daltonics, Fremont, CA). The double disc synergy test (DDST) with amoxicillin clavulanic acid (10µg), cefotaxime (30 µg), and ceftazidime (10µg) was performed to detect the presence of ESBL bacteria. Furthermore, a disk diffusion test with meropenem disc (10 µg) was performed to screen the presence of carbapenemases-producing bacteria. All phenotypically ESBL-positive *Escherichia coli* isolates were tested for $bla_{\text{CTX-M}}$ and bla_{TEM} genes with a PCR Multiplex.

Results

A total of 11 dairy farms from Lombardy, 4 farms from Piedmont, and 1 from Emilia Romagna were enrolled. One hundred and thirty-three calves were enrolled in the study, and 122/133 were phenotypically positive for ESBL bacteria. In particular, 28 out of 30 calves were positive for ESBL in Piedmont, 84 out of 93 samples were positive in Lombardy, and 10 out of 10 were positive in Emilia Romagna.

The number of detected ESBL bacteria species was variable depending on the sample. In 96 samples only one ESBL bacterial species was detected, followed by more than one species detected in 26 samples. In 11 samples there was no bacterial growth on ESBL selective media. The most detected ESBL-bacteria species was *E. coli* in 107 fecal samples, followed by *Acinetobacter baumannii, Klebsiella pneumoniae*, and Stenotrophomonas maltophilia, detected respectively in 20, 19, and 1 fecal samples. All these isolates were phenotypically ESBL-producing bacteria. Only 1 isolate resulted in being resistant to meropenem.

96 ESBL-*E. coli* were tested for $bla_{\text{CTX-M}}$ and bla_{TEM} genes. The number of samples positive to both genes was 44, while the number of samples positive only to blaCTX-M was 6,

and the number of E. coli only positive for *bla*$_{TEM}$ was 17. The number of negative samples to the PCR was 29.

Conclusions

The results showed a high prevalence of EBSL-producing bacteria, 91,7% phenotypically and 69,8% genotypically, in neonatal dairy calves' feces in Northern Italy.

The ESBL genes were distributed similarly across all three regions when considering the co-occurrence of both genes. However, when looking at the individual positivity of each gene, only the Piedmont region showed positivity for the *bla*$_{CTX-M}$ gene, while the other two regions showed positivity for the *bla*$_{TEM}$ gene. The prevalence and the species of ESBL-producing isolates detected in this study are comparable to other European studies.

The singular resistance of the sample to meropenem could potentially be attributed to the avoidance of this particular antimicrobial class in veterinary medicine. Furthermore, the species of the isolate, *S. maltophilia*, could also be a contributing factor due to the high resistance rate of β-lactams, including cefuroxime, cefoxitin, imipenem, and meropenem, as a result of β-lactamase production. This is a preliminary study, and further investigation will be performed, increasing the sample size and testing other genes responsible for ESBL.

1567 - Comparative efficacy of stannous fluoride and copper sulfate footbath solutions for the prevention and treatment of digital dermatitis in lactating dairy cows

Author: Tessa Marshall , Peter Constable, Alistair Kenyon

Objectives
To determine whether a novel footbath solution containing stannous fluoride (SF) was superior to a footbath containing copper sulfate (CS) solution for the prevention and treatment of digital dermatitis (DD) in dairy cattle.
Material and methods
Two prospective randomized clinical trials were conducted using convenience sample populations and a positive control treatment (CS). Study 1 was conducted over 4 weeks in Missouri and involved 34 lactating Holstein-Friesian cows with active DD lesions in at least one hind foot. Cows in group SF walked through a footbath containing a proprietary formulation of SnF_2 once weekly, whereas cows in group CS walked through a 5% $CuSO_4$ footbath once daily for five days each week. Study 2 was conducted over 8 weeks in California and involved 40 lactating Holstein-Friesian cows with active DD lesions in at least one hind foot. Cows in group SF walked through a footbath containing a proprietary formulation of SnF_2 for three consecutive days then once a week for the following 7 weeks. Cows in group CS walked through a 5% $CuSO_4$ footbath three times each week for 8 weeks. Data collection included lesion type, lesion area, locomotion score, and pain score. Lesion type was categorized as active (M1, M2), inactive (M3, M4), or absent (M0). Lesion area was determined as the mean of three measurements using a digital photograph of the hind foot and ImageJ software. Statistical analysis was conducted by an investigator that was masked to treatment assignment.
Results
Digital dermatitis was actively transmitted in study 1, and lesion area and locomotion scores were lower during the study in group SF than group CS. In contrast, DD was not actively transmitted in study 2, and lesion area and locomotion scores during the study were similar in groups SF and CS. Stannous fluoride delayed the development of active DD lesions in study 1 compared to copper sulfate, with a lower relative risk (0.57, $P < 0.001$) of a hind foot developing an active DD lesion over 28 days. For comparison, the cure rate of active DD lesions was lower in group SF than CS; the relative risk of a hind foot with an active DD lesion being cured in group SF was lower than group CS in study 1 (0.83, $P = 0.042$) and study 2 (0.90, $P = 0.020$).
Conclusions
Walking cows through a stannous fluoride footbath once per week in a herd undergoing active transmission of infection was more effective in preventing active DD lesions, but less effective in treating active DD lesions, than walking cows through a copper sulfate footbath four times per week. The novel SnF_2 footbath solution shows promise for controlling DD in dairy herds that wish to use a less labor-intensive footbath regimen than that commonly used for 5% $CuSO_4$ solutions and are interested in limiting environmental accumulation of copper.

1569 - Presence of antibodies against BVDV and IBR (BoH1) virus in blood serum from dairy family farms in Pontal do Paranapanema, State of São Paulo, Brazil.

Author: Mario Augusto Reyes Alemán, Jeferson Carvalho , Adriana Romaldini, Ander Yuren , Lilian Gregory

Objectives
Evaluation of antibodies in the blood serum of dairy farmers bovine family productions of the Pontal do Paranapanema region, State of São Paulo, Brazil.
Material and methods
A total of 616 blood serum samples were obtained from dairy cattle from family farms in the Pontal do Paranapanema region, State of São Paulo, Brazil. The samples were obtained by puncture vein of the caudal vein using a vacutainer® tube without anticoagulant to obtain blood serum. After collection, blood samples were centrifuged to obtain blood serum and subsequently aliquots, for further processing at the Laboratory of Viruses of Bovids of the Instituto Biológico de São Paulo. The sera were tested by the virus-neutralization plate technique, to find out the presence of antibodies to BVDV and IBR.
Results
After doing the virus-neutralization test we obtained the following results: 61.53% (379/616) were reactive (positive) the presence of antibodies against IBR and 34.58 (213/616) were reactive (positive) the presence of antibodies against BVDV.
Conclusions
It concluded that in family dairy production in the Pontal do Paranapanema region, there is a greater presence of antibodies against IBR than in BVDV, this can cause great losses in the reproduction of dairy acts in the region, thus causing a low rate of replacement of animals. Studies in this area should continue to be carried out to improve actions in the region of the Paranapanema point, as one of the causes of the spread of these diseases and the lack of information and technical assistance in the region.

1580 - Antimicrobial resistance in pathogens isolated from raw milk and fresh goat cheese.

Author: Israel Daniel Ricardo González, Laura Hernandez Andrade, Edith Rojas Anaya, Gary Gacia Espinosa, Susana Elisa Mendoza Elvira

Objectives

The objective was to identify potentially pathogenic microorganisms, as well as their susceptibility to antimicrobials, from samples of milk and cheese produced on family farms in the northern, central and southern regions of Mexico.

Material and methods

Two farm units from the state of Durango, two from the state of Querétaro and two from the state of Campeche were sampled. Samples of raw milk were taken and in the case of cheeses, 2 to 3 samples of cheese made from the sampled milk tank were taken. The samples were prepared according to NOM-110-SSA1-1994 and were plated on Blood and MacConkey agar. The susceptibility profiles of the isolated strains to select antimicrobials were determined by the Kirby-Bauer method, using for Gram positives: Ampicillin (AM), Cefotaxime (CFX), Ciprofloxacin (CPF), Clindamycin (CLM), Erythromycin (E) , Penicillin (PE), Tetracycline (TE), Cephalothin (CF), Dicloxacillin (DC), Gentamicin (GE), Sulfamethoxazole Trimethoprim (STX) and Vancomycin (VA), while for Gram negatives the following were used: Ampicillin (AM), Carbenicillin (CB), Cephalothin (CF), Cefotaxime (CFX), Ciprofloxacin (CPF), Chloramphenicol (CL), Nitrofurantoin (NF), Amikacin (AK), Gentamicin (GE), Netilmicin (NET), Norfloxacin (NOF) and Sulfamethoxazole Trimethoprim (STX). Descriptive statistics were performed on the data, while the Mann-Whitney U test was used to compare the resistance profiles between microorganisms isolated in raw milk and fresh cheese.

Results

Staphylococcus aureus was isolated from raw milk in 5 of the 6 establishments sampled, in all 6 strains of Coagulase Negative *Staphylococcus* (CNS) were isolated, in all samples various species of *Streptococcus* were isolated, in 1 *Enterococcus faecalis* was isolated and in 2 *Listeria ivanovii* and *Listeria welsmeri* and *Bacillus cereus*. In the case of Gram-negative pathogens, *E. coli* was isolated in all samples, in three P*seudomonas aeruginosa*, in two *Proteus vulgaris* and *Proteus mirabilis,* and in one K*lebsiella pneumoniae* and *S. aureus* was isolated in all cheeses, as well as various species of *Streptococcus*. For Gram-negative bacteria, *E. coli* was isolated in all samples, *Proteus mirabilis* was isolated in 4 samples, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter aerogenes* were isolated in 3 samples. 50% of the Gram-positive microorganisms from raw milk presented resistance to antibiotics such as AM, CFX, CLM, PE, TE, CF and DC, all *Staphylococcus, Streptococcus* and CNS presented 100% resistance to PE and the isolated strain of *E. faecalis* was resistant to VA. Of the Gram-negative bacteria isolated from raw milk, 71% of the microorganism's showed resistance to AM, CB, CF, CF, NF and GE; 100%, *P. aeruginosa* was resistant to AM and 80% to AK. 83% of the Gram-positive pathogens isolated from cheeses were resistant to AM, CFX, PE, TE, CF and DC; S*treptococcus dysgalactiae and E. faecalis* were resistant to AM, as well as 100% of *S. aureus, S. dysgalactie, S. agalactiae* and SCN were resistant to PE. Gram-negative microorganisms, 75% showed resistance to AM, CB, CPF and GE; 100% of the *E. coli* were resistant to AM and 80% of the *P. aeruginosa* were resistant to AM, NF and

NOF. When comparing the general resistance averages for bacteria obtained from milk versus those obtained from cheese, no significant difference was found.

Conclusions

n this study, the presence of bacteria considered pathogenic and causing food-borne diseases was evidenced in fresh goat cheeses as well as in the milk that gave rise to them produced in family systems in Mexico, highlighting the impact of the lack of good practices. livestock and manufacturing in these productive units, manifesting itself as contamination due to hygienic-sanitary deficiencies during the productive chain, which implies a risk to public health combined with the growing profile of resistance to antimicrobials presented by this type of microorganisms.

This study was funded by PAPIIT IN203522 and Research Chair CI2216 FES-Cuautitlan UNAM, as well as with self-generated resources from the animal bacteriology laboratory. CENID SAI INIFAP-SADER

1582 - Biofilm formation and phenotypic determination of Extended Spectrum Beta-lactamases (ESBL) in Klebsiella pneumoniae strains from cases of bovine mastitis.

Author: Miguel Ángel Blanco Ochoa, Israel Daniel Ricardo González, Laura Hernandez Andrade, Marco Antonio Santillán Flores

Objectives

The objective of this work was to determine the formation of biofilm, as well as to phenotypically determine the presence of ESBL through antimicrobial sensitivity tests in strains of *K. pneumoniae* from cases of bovine mastitis.

Material and methods

The present work was carried out from 10 samples of clinical mastitis cases sent to CENID SAI INIFAP, which upon receipt were sown with blood agar and MacConkey, identification was carried out by means of smears, Gram stain and traditional biochemical tests. A screening test was performed on the strains identified as *K. pneumoniae* by measuring the Kirby-Bauer technical inhibition zones and selecting those that had inhibition zones less than 27 mm for Aztreonam, 22 mm for ceftazidime and 25 for Ceftriaxone. The detection of ESBL was carried out by the Hodge method, which consisted of preparing a suspension of the *E. coli* ATCC 25922 strain, with turbidity equivalent to tube No. 0.5 on the McFarland scale, this suspension was inoculated on a Mueller Hinton agar plate. One plate was used for each disc of Cefotaxime (CTX), Ceftriaxone (CRO), Aztreonam (ATM) and Ceftazidime (CAZ), where a 2 cm streak was made of the problem strains results of the screening tests, from the center to outside the disk. The presence of the enzyme was identified by observing a deformation of the inhibition zone of the *E. coli* ATCC 25922 strain to the disc with the slit-shaped substrate. The plate biofilm formation test was performed in triplicate by placing 100 µl of BHI broth and 100 µl of a standardized *K. pneumoniae* solution in the 0.5 McFarland tube. As a positive control, a strain of S. aureus strain Cowan (CSA) was used Plates were stained with 0.1% crystal violet and read with an ELISA plate reader at a wavelength of 492nm. Data analysis was performed using an ANOVA and a Tukey test, using the IBM SPSS Statistics 22.0 statistical program.

Results

During the screening test, only 4 of the 10 *K. pneumoniae* samples were suggestive of the presence of ESBL, representing 40% of the samples analyzed, of which only two (20%) were positive to the Hodge test. On the other hand, all strains could form biofilm, obtaining absorbance values of 0.488 ± 0.305, which turned out to be statistically different ($p < 0.05$) to the negative control used, which obtained an absorbance of 0.019. It is important to mention that the four strains suspected of ESBL, including the two that were positive to the Hodge test, obtained higher absorbance values (0.856 ± 0.118) compared to the rest of the strains evaluated (0.275 ± 0.073), indicating a greater capacity for biofilm formation.

Conclusions

Finding 2 strains of *K. pneumoniae* with the presence of these ESBLs in cases of bovine mastitis is a warning sign to raise awareness of the possible consequences of the dissemination of these resistance genes among production animals and their possible effect on public health. Although there is not enough information about the

identification of this type of resistance mechanisms in bacteria of the *Klebsiella* genus isolated from cases of bovine mastitis, these have already been reported in broiler chickens and pigs. The importance of these ESBLs and their presence in production animals lies in the possible transmission and spread of these genes through zoonotic infections through their byproducts. The above highlights the importance of the emphasis on mastitis prevention activities and the correct use of antimicrobial therapies to reduce the prevalence of intramammary infections caused by Enterobacteriaceae.

This study was funded by self-generated resources from the animal bacteriology laboratory. CENID SAI INIFAP-SADER

1590 - In vitro microbiological evaluation of six sealants with different active ingredients (iodine, chlorhexidine, and latent active chlorine) against pathogens commonly isolated in cases of bovine mastiti

Author: Lysett Corona-Gómez, Laura Hernández-Andrade, Israel Daniel Ricardo-Gónzalez, Israel Adair Cabrera Contreras, Ana Elvia Sanchez Mendoza

Objectives

Compare the in vitro microbiological effectiveness of different sealants against different pathogens commonly isolated from cases of bovine mastitis.

Material and methods

6 sealants were used with the following active ingredients: 1% iodine (Y1), 1% iodine with aloe vera (Y1A) and 0.5% iodine (Y05); 2 based on chlorhexidine: Chlorhexidine Digluconate 0.5% (DC) and Chlorhexidine Gluconate 0.4% (GC) and latent active chlorine derived from Sodium Troclosene (TS).

The sealants were challenged with 22 strains of microorganisms isolated from cases of bovine mastitis, distributed as follows Gram negative microorganisms: 3 strains of *Escherichia coli* (ATCC 8739), 3 of *Klebsiella pneumoniae* (ATCC 700603) and 3 of *Pseudmonas aeruginosa* ; Gram positive microorganisms: 3 strains of *Staphylococcus aureus* (1 ATCC BAA976), 3 of *Streptococcus dysgalactiae*, 3 of *Streptococcus uberis* and 3 of *Bacillus cereus (*ATCC 14579*)*; yeasts: 3 strains of *Candida albicans* (ATCC 14053).

The agar diffusion technique was used using stainless steel penicylinders placed on the Mueller Hinton agar plates inoculated with the bacterial dilution of 10^8 CFU/mL of each of the microorganisms in triplicate, subsequently 250 µL of the products to be evaluated were added; A commercial solution of benzalkonium chloride tincture (0.250 mg/100 mL) was used as a positive control, and sterile saline solution was used as a negative control.

The inhibition zones were measured, and the diameters obtained were compared with the values suggested by Hernandez et.al. 1999 to determine the degree of activity: no inhibitory activity (8-16 mm), regular activity (17-22 mm), good or greater (23-30 mm) and excellent (30 mm), additionally descriptive statistics were applied using the IBM SPSS program.

Results

In the case of Gram-negative bacteria, the sealants that did not show activity (8-16 mm) were Y1 for strains of *K. pneumoniae* and *P. aeruginosa*; DC and TS for *P. aeruginosa*, while Y1A and Y05 for all the microorganisms evaluated. The sealants that showed regular activity (17-22 mm) were: DC and TS for *E. coli* and *K. pneumoniae* strains, Y1 only for *E. coli* strains and GC for all strains evaluated.

For Gram-positive bacteria, the sealants that did not show activity (8-16 mm) were Y1 for *S. dysgalactiae*; Y05 and TS for *S. uberis*, *S. dysgalactiae* and *B. cereus*; DC for *S. dysgalactiae* and B. cereus and YIA for all Gram-positive microorganisms evaluated. The sealants that showed regular activity (17-22 mm) were: Y1 for *S. aureus*, *S. uberis* and *B. cereus*; Y05 and TS for *S. aureus* and *B. cereus*; DC for *S. aureus* and *S. uberis* and GC for all microorganisms evaluated.

When evaluating the activity of the sealants against yeasts, it was found that Y1A and TS did not show activity (8-16 mm) against them, Y1 and Y05 showed a regular inhibitory activity (17-22 mm) and DC and GC showed a good activity (23-30 mm).

The averages of the inhibition zones obtained in the controls were the following: 29.62 mm for the positive control (good inhibitory activity) and 0 mm. For the negative control.

Conclusions

Considering the values suggested by Hernandez et.al. 1999, the sealants did not show good or excellent activity for Gram-negative and Gram-positive bacteria, for yeasts the classification of good was achieved. In general, the sealants showed regular activity against the microorganisms evaluated. The microorganisms with the highest frequency of resistance were *K. pneumoniae, P aeruginosa, S. dysgalactiae* and *S. uberis*.

The selection of sealants is essential for the control of bovine mastitis, the choice will depend on the status of the barn as well as the infectious or environmental bacteria that are causing problems within it, the cost of the products also influences your choice, so it is important to evaluate all the factors and their cost-benefit.

1595 - Molecular and bacteriological diagnosis of lesions suggestive of clostridial infection in domestic ruminants

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Objectives
Several species of genus *Clostridium* are responsible for necrotic lesions in multiple animal species, including humans.
To describe some necropsy cases of domestic ruminants with clostridiasis-suggestive lesions compared with bacterial isolation and PCR analysis.
Material and methods
Samples of fifty-seven necropsy cases of domestic ruminants showing lesions in intestine, liver, kidney and/or muscle, were paraffin-embedded, then HE and Gram stained. Some organ samples were bacteriologically diagnosed. Subsequently, genomic DNA was extracted from those paraffin-embedded sections and *Clostridium perfringens* was identified by PCR amplification of a 279 bp fragment of the 16S rRNA gene.
Results
Clostridium isolation was achieved in ten cases, involving single or multiple species (*perfringens, novyi, chauvoie, septicum*). Other different bacteria genera were identified in nine cases. Twenty-nine samples were PCR-positive for *C. perfringens*. Additionally, the gen *cpa* which codifies the alfa-toxin, was amplified in three cases. Isolation-PCR correlation was identified in only five cases.
Conclusions
The main diagnostic challenge of clostridiasis is the difficulty establishing causality for strains and toxins that can also be found in healthy animals. Combined infections of *Clostridium* spp with other pathogens are frequently described.
Keywords: *Clostridium*, domestic ruminant, bacteriologic analysis, PCR

1599 - Acute phase proteins following simultaneous vaccination of lumpy skin disease and foot and mouth disease

Author: Jiyeon Kim, Woojae Choi, Hyoeun Noh, Leegon Hong, Eunwoo Chun, Eunkyung Kim, Danil Kim, Younghye Ro

Objectives

Foot and Mouth Disease (FMD) and Lumpy Skin Disease (LSD) are classified and managed as contagious animal diseases type I in South Korea. After the first outbreak of FMD in 2010, FMD vaccination was conducted twice a year (April and October), and the LSD vaccines were vaccinated nationally in October 2023 following the first outbreak of LSD. However, Korean livestock farms tend to be reluctant to administer a new vaccine because they have previously experienced adverse reactions such as abortion and decreased productivity probably due to the acute immune response caused by FMD vaccination. Therefore, this study was conducted to estimate the characteristics of possible vaccine adverse reactions by identifying the acute immune response of LSD vaccine.

Material and methods

Seventeen cows were used in the experiment. Group 1, consisting of 5 cows, was administrated intramuscularly with the FMD vaccine (Himmvac FMD, KBNP Inc., Korea), and Group 2, consisting of 6 cows, was administrated subcutaneously with the LSD vaccine (Lumpyvac, VETAL Animal Health Products Inc., Turkey). Group 3, consisting of 6 animals, was treated simultaneously with FMD and LSD vaccines. Blood samples were collected from the coccygeal vessel with a heparin tube before vaccination and on 3, 6, and 10 days after vaccination. Plasma was separated immediately after blood collection and stored at -70°C until analysis. Serum amyloid A (SAA) and haptoglobin (HP) were used as acute phase proteins (APP), and measurements were taken according to the respective manufacturer's instructions. Two-way repeated measure ANOVA was used for statistical analysis, followed by multiple comparisons with Bonferroni t-test.

Results

In Group 1, SAA peaked on day 3 of administration ($p < 0.001$) and then returned to its original level. In Group 2, the average concentration of SAA gradually increased and tended to be statistically high on day 10 after administration ($p = 0.063$). In Group 3, it peaked at day 3 post-vaccination ($p < 0.001$) and remained high at day 10 ($p < 0.01$). On day 3 after vaccination, group 3 had significantly higher SAA concentration than group 2 ($p < 0.001$), but there was no significant difference with group 1. On day 10 of vaccination, the average SAA concentration of group 3 was significantly higher than that of groups 1 and 2 ($p < 0.001$).

For HP, only in GROUP 3, HP concentration was significantly higher from 3 to 10 days after vaccination ($p < 0.001$). When compared between groups, the average HP concentration at day 3 was significantly higher in Group 3 compared to Group 2 ($p < 0.01$) and tended to be higher than Group 1 ($p = 0.064$). On days 6 and 10 of vaccination, the average HP concentration in group 3 was significantly higher than in groups 1 and 2 ($p < 0.001$).

Conclusions

As previously reported, the concentration of APPs in Group 1 peaked on day 3 after administration, and then gradually decreased, reaching the pre-administration level 10

days post-vaccination. In Group 3, the average concentration of APP tended to be high on day 10 after LSD vaccination, not immediately after administration. In Group 3, which was simultaneously administrated with FMD and LSD vaccines, both an increase on day 3 due to FMD and an increase on day 10 due to LSD were observed. This difference is thought to be due to differences in the adjuvant of each vaccine. In the FMD vaccine, which is an inactivated virus vaccine, paraffin oil is added as an adjuvant to increase immunogenicity. In contrast, the LSD vaccine is an attenuated virus vaccine, and a combination of lactalbumin 96 hydrolysate and sucrose is used as an adjuvant. The adjuvant used in the FMD vaccine induces an immune response by increasing monocytic exudation, which causes an inflammatory response similar to a pathogen infection. The LSD vaccine adjuvant is used to stabilize antigens and has the effect of maintaining antigenic stabilization for a long period. In previous studies, it was known that simultaneous administration of LSD-like sheep pox vaccine and FMD vaccine had no problem with antibody formation. However, further research is needed on the continuation of the acute immune response and the long-term adverse effects caused by simultaneous vaccination of the two vaccines.

1600 - Meningitis and enterocolitis associated with bovine coronavirus in Korean native calf

Author: Hyung-Chul Cho, Youngjun Kim, Kyoung-Seong Choi

Objectives

Bovine coronavirus (BCoV) is a pneumoenteric virus that can infect both digestive and respiratory tracts of cattle, resulting in enormous economic losses. Meningitis in calves often occurs with sepsis and is primarily caused by bacterial infection, but also reported by viral infection. This case presents BCoV-associated viral meningitis in a Korean native calf with hematochezia.

Material and methods

A 3-week-old Korean native calf exhibited hematochezia for five days and developed dehydration, depression, hypothermia, and exertional dyspnea. The patient was in a semicomatose state with opisthotonos and showed absent menace and nasal tactile response but with a weak response for pain stimuli. The calf died two days after the onset of clinical signs. During the necropsy, blood, cerebrospinal fluid (CSF), and tissue samples including brain, lungs, kidney, rumen, abomasum, mesenteric lymph node (MLN), and small and large intestines were collected and used for DNA and RNA extraction to detect pathogens associated with meningitis. Only BCoV was identified and positive samples were directly sequenced. The remaining tissue samples were subjected to histopathological examination and immunohistochemistry (IHC).

Results

The CSF results of this calf revealed lymphocytic pleocytosis (lymphocyte 84.4% and monocyte 15.6%), increased protein concentration (2 g/L at antemortem) and L-lactate (8.68 mmol/L), and plasma glucose concentration (1.1) ratio. Except for the blood and CSF, BCoV was detected in all tissues examined including brain. *E. coli* was also identified in brain, lung, MLN, and rumen. The BCoV sequences identified in this calf showed 98.1%–100% identity to each other and exhibited 97.7%-99.5% identity with those of diarrheic calves reported in Korea. There were no histopathological abnormalities in the brain and the most prominent histopathological findings, such as necrotizing hemorrhagic suppurative bronchopneumonia, were found in the lung. By IHC analysis, the BCoV Ag was not detected in the brain, but was identified in the rumen, small and large intestines.

Conclusions

Although BCoV was not detected in the CSF, these findings in the CSF may be good indicators for diagnosing viral meningitis. Here, we report for the first time a new clinical sign, namely BCoV-induced meningitis in cattle. More studies on the role of BCoV as a potential neurologic, enteric, and respiratory pathogen are warranted.

1626 - ETIOLOGICAL AGENTS ISOLATED FROM NEONATAL CALF DIARRHEA (NCD) OUTBREAKS IN EMILIA-ROMAGNA REGION (ITALY) DURING 2020-2022

Author: Camilla Torreggiani , Alice Prosperi, Giovanni Pupillo, Chiara Anna Garbarino, Gianluca Rugna, Roberta Manfredi, Valentina Gabbi, Andrea Luppi

Objectives

Neonatal calf diarrhea (NCD) is the most important cause of death in newborn calves. It is, also, responsible for serious economic losses due to mortality, poor weight gain and increased costs for antimicrobial treatments. NCD has a multifactorial etiology, in which viruses, bacteria, protozoa and management factors (housing, feeding, hygienic conditions) play a crucial role. NCD is attributed to infectious and non-infectious causes. Among infectious causes, the main pathogens are: *Cryptosporidium parvum,* viral pathogens as bovine rotavirus (BRV), bovine coronavirus (BCoV) and enterotoxic *Escherichia coli* (ETEC). The prevalence of the different etiological agents varies with the calfs' age and co-infections are frequently reported.

The aim of this study is to determine the prevalence of the main infectious agents of NCD, in diarrheic calves from 1 to 21 days of age, from dairy herds in Emilia-Romagna region (Italy) during 2020-2022.

Material and methods

Between the years 2020-2022, 672 calves under 22 days of age from 598 Italian dairy herds were sent to Emilia-Romagna diagnostic laboratories of the Istituto Zooprofilattico Sperimentale of Lombardia and Emilia-Romagna for diagnostic purposes.

Feces and intestines collected during necropsies were processed using standard procedures for isolation and characterization of *E. coli*, in brief, samples were plated on blood agar and Gassner agar and aerobically incubated for 24–48 h at 37°C.

A multiplex PCR for the detection of fimbrial and toxin genes, including those encoding for F4 (K88), F5 (K99), F6 (987P), F18, F41, LT, STa, STb and Stx2e was performed for *E. coli* colonies. Isolates encoding at least one of the investigated enterotoxins were classified as ETEC. When strains with the same virotype were detected within the same outbreak, they were considered only once for prevalence calculations.

Fecal samples were submitted for real time PCR for the detection of group A bovine rotavirus (BRV) and bovine coronavirus (BCoV). Stool samples were examined for confirmation of the presence of *Cryptosporidium parvum* oocysts by modified Ziehl–Neelsen staining method.

Results

A total of 498 *E. coli* strains were isolated and subsequently genotyped by PCR. Among these, 101 strains were classified as ETEC and showed a prevalence of fimbriae and toxins distributed as follows: F5/K99 F41 (50.5%), F5/K99 (39.6%), F4/K88 (7.9%), F41 (0.9%), F6 (0.9%), STa (91.1%)**.** The ETEC virotypes most commonly detected were F5 F41 STa and F5 Sta, found in 50.5% and 37.6% of cases, respectively.

ETEC strains were found in 17.2% of the 598 NCD-affected farms investigated.

Considering other etiological agents investigated, BRV, BCoV and *C. parvum* showed prevalences of 22.2%, 20.2%, 32.3% respectively.

The most frequent co-infections were: BRV and *C. parvum* (10.2%), followed by BRV and BCoV (8.5%) and finally by BRV and ETEC (3.8%).

Conclusions

The present study examines the prevalence of the main infectious agents isolated from NCD cases in the Emilia-Romagna region (Italy) during 2020-2022. When considering ETEC, the fimbrial F5 gene was the most prevalent, which is consistent with other European studies. Regarding ETEC F4 strains, these were detected in the present study, but, as other authors report, they have been widely described in the pig, and their role in calves is not yet completely clarified.

According to previous European investigations, BRV is the most frequently detected agent among viral NCD pathogens. *C. parvum* was found in 32.3% of cases. When compared to other European studies, results may vary, but these results need to be interpreted carefully since the sampled population and tests may differ between studies.

In addition, the interaction between different pathogens the severity of enteric disease, as evidenced by some studies in which mixed infections were much more frequent in diarrheal animals compared to healthy animals.

NCD is still one of the leading health concerns in calves. Control measures begin with a recollection of historic data, identification of affected animals, and the determination of the underlying etiology, whether infectious or non-infectious, using an adequate diagnostic approach. Biosecurity and direct prophylaxis are key factors in infection control.

Monitoring pathogens on the farm and applying the correct diagnostic approaches, provides information and is the preferred strategy for infection control and prevention.

1631 - Ureaplasma diversum infection causes polyarthritis and interstitial pneumonia in beef calves in Uruguay

Author: Cintia Queiroz-Machado, Mizael Machado , Rafael Martinez, Sol Andres, Martín Fraga, Marina Berón, Alejo Menchaca, Franklin Riet-Correa

Objectives

Ureaplasma diversum is a Mollicute wall-less bacterium that possesses the enzyme urease and is associated with reproduction and economic losses in cattle-producing farms. It is an opportunistic pathogen that colonizes the respiratory and genital tract of cattle and can lead to reproductive disorders such as vulvovaginitis, endometritis, mastitis, and placentitis. Rarely, fetal alveolitis and fetal polyarthritis may result in abortion or the birth of weak calves and neonatal death. The main form of transmission is through natural reproduction, but horizontal and vertical transmission, as well as transmission through artificial insemination and embryo transfer, may also occur. The presence of *Ureaplasma* spp. in different secretions of healthy animals makes it difficult to establish control strategies against the infection in cattle herds. The main objective of this study is to describe a cluster of bovine neonatal deaths caused by *Ureaplasma diversum* in beef cattle in Uruguay.

Material and methods

A breeding farm reported an increase of 7-8% in the percentage of losses of calves (abortion and neonatal death), reaching up to a mortality rate of 13.5% of calves originated from natural breeding and a 14.5% mortality rate of calves originated from artificial insemination. Previous rates of calf losses were usually around an average of 6.5% on the same farm. Most of the affected calves were born weak and would end up dying in one to three days, even though abortion and stillbirths also occurred. Frequently, the cows would present retained fetal membranes after the birth of the affected calves or after the abortion. In a three-day period, six calves were sent for necropsy examination to investigate the cause of death. At that time, there were 1450 pregnant cows, 330 fresh cows, and 40 dead calves. Four of the necropsied calves died naturally, while two were euthanized due to the poor prognosis. All the examined calves were 1 to 2 days old. Post-mortem examination study was conducted, while several fresh tissue samples were taken for molecular studies to investigate the most common infectious causes of bovine abortion and neonatal death, including *Neospora caninum*, *Leptospira* spp., *Chlamydia abortus*, *Chlamydia pecorum*, *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Ureaplasma diversum*, and bovine viral diarrhea virus.

Results

At gross examination, four out of six calves exhibited mild polyarthritis characterized by the presence of fibrinous exudate in two or more different articular locations, with the carpal and tarsal joints being the most frequently affected joints. One of the calves with polyarthritis also exhibited a diffuse mild deposition of fibrinous material in the external surface of the organs in the abdominal cavity (polyserositis). Histologically, the synovial membranes of these four calves exhibited mild to moderate lymphohistiocytic infiltration with rare neutrophils and a variable amount of fibrin deposition. Another frequent finding was mild to moderate interstitial lymphohistiocytic pneumonia with occasional neutrophils (3/6), lymphohistiocytic myocarditis (2/6), lymphohistiocytic

hepatitis (2/6), neutrophilic and thrombotic cystitis (1/6), were also observed. Molecular tests resulted positive for *Ureaplasma diversum* in the lungs of three different calves.

Conclusions

We describe the occurrence of an uncommon cluster of bovine neonatal deaths in a breeding farm associated with *Ureaplasma diversum* leading to lesions rarely reported. It is necessary to investigate the presence of this agent in other cattle farms in Uruguay causing reproductive losses or in healthy cattle.

1633 - A severe case of papillomatosis in pelvic and thoracic limbs as causes of early replacement of a Holstein heifer in a dairy cattle herd

Author: Juliana Melo Pankratz, Rüdiger Daniel Ollhoff , Ana Carolina Martins Bock, Diógenes Adriano Duarte Santana, Isabelle Rumika Ollhoff

Objectives

Report an unusual clinical case of severe papillomatosis in a Holstein heifer at the Gralha Azul farm hospital, Pontifícia Universidade Católica do Paraná (PUCPR), located in the municipality of Fazenda Rio Grande, state of Paraná, southern Brazil.

Material and methods

A 13 old months Holstein heifer, weighing 349 kg, body condition score 2 (1-chachetic to 5-obese) exits production due to delayed body development and brought to the hospital due to several hyperkeratotic papillomas concentrated mainly on all four limbs. It was the first heifer with this specific clinical appearance in the history of the farm founded in 1999, although non severe papillomatosis is common in the herd. The first signs of papillomatosis were noted by the farmer in September 2022. In March of 2023 the heifer came to the hospital and at first clinical examination cardiac, respiratory, ruminal function and temperature were all normal. The majority of the papillomas were covering both forelimbs and both hindlimbs following the same macroscopic pattern of hiperqueratotic grape-shaped appearance. Of the right forelimb, 50% of the skin between caudal carpometacarpal and the interdigital joints was affected. The major clusters close to caudal and lateral metacarpo-phalangeal joint were visible in lateral, caudal and frontal view, with some smaller lesions in caudal view of the carpus. Of the left forelimb, 30% of the skin between caudal carpometacarpal and the interdigital joints was lesioned. The major cluster was in the caudal region of the interdigital region visible from the lateral and caudal view. Of the right hindlimb, 20% of the skin between tarsometatarsal and the interdigital joints was affected. From the lateral view of the limb, it was possible to observe smaller, spaced warts with a pedunculated appearance, irregular surface, and hard consistency in the distal metatarsal region from the lateral view. Of the left hindlimb, 10% of the lateral skin between tarsometatarsal and the interdigital joints was affected. In the lateral view, the appearance consisted of papillomas in various sizes, spaced approximately 2 cm apart, with a pedunculated appearance. Isolated warts were found in the lateral view of the abdominal areas, the scapular region, as well as the submandibular and craniofrontal regions. The following therapeutic approaches were employed: autogenous papilloma vaccine with a 5 mL subcutaneous application and revaccination every 7 days for 4 consecutive weeks, auto-hemotherapy with a 20 ml intramuscular application of venous blood, and levamisole (3.5 mg/kg s.c.) once in a week for 3 times, and finally, the surgical excision (pulling out) of the papillomas. Tissue samples were sent to the virology laboratory for virological identification.

Results

Unfortunately, even after nine months, none of the treatments proved effective. The heifer continues to exhibit disseminated warts on its limbs and body. Actually, the animal lost weight (279 Kg; BCS 1,5), and started biting some papillomas due to the installation of a myiasis beneath the papillomas. This was controlled by mechanically removing larvae and systemic use of ivermectin 50 mcg/kg SID s.c. Resulting skin wounds

were treated with topic zinc oxid ointment after prior disinfection. Virology results were not available until abstract closure.

Conclusions

Even though all the used treatments are cited in literature to be effective in controlling papillomatosis, they proved ineffective in this case. The missing scientific information about pathogenesis of papillomatosis, specifically the incapacity to fulfill the Koch's postulates, remain challenging for the development of universal adoptable effective therapies. Cattle with clinical signs of papillomatosis for more than 6 months have a poor prognosis. The prevalence of papillomatosis as a reason for early replacement of heifers is unknown.

1641 - Use of the beta-lactamase-resistant cloxacillin for the dry-cow therapy: Minimum Inhibitory Concentrations (MICs) for Streptococcus uberis in Germany

Author: Thomas Breuer, Marcus Klawitter, Ulrich Löschner, Ansgar Busch, Torsten Steppin, Maria Elena Vergara Hernandez, Luis Leon

Objectives

Due to changes in EU regulations, the blanket use of antibiotic dry-cow therapy (ADCT) should not be longer the norm in dairy farms (Regulation (EU) 2019/6). In Germany, the use of commercial fixed antibiotic combinations at dry-off is very extended. The new legislation limits the use of this kind of preparations adding a factor that increment the annual antibiotic-use-benchmarking for farms.

Veterinarians are looking for effective alternatives. The beta lactamase-resistant penicillins like cloxacillin have been used in the treatment of mastitis. The medications are commonly mono-products, and the compound is considered by the EMA as prudent use antibiotic (first line treatment). Cloxacillin is consider as anti-staphylococcal penicillin with high effectivity against pathogens causing often subclinical mastitis. There was no significant difference between treatments with Cloxacillin and the 1st generation cephalonium on quarter cure rates for new infections, for chronic infections and for infections with *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus uberis* at dry off (Shephard et al: 2004): The present study analyzed the MIC situation of cloxacillin for *S.uberis* in Germany.

Material and methods

Randomly selected quarter milk from mastitis cases on commercial dairy farms from different regions in Northern Germany were sampled. The samples were analyzed at the Milk Quality Laboratory of the University of Hannover using broth dilution test. S. uberis Identification was based on morphology, hemolysis patterns, esculin hydrolysis, gram staining, catalase, OF-test, sub cultivated on modified Rambach agar-β-d-galactosidase-positive cocci (Watts et al. 1993) and PCR. The test is considered as the gold standard because it provides reproducible, quantitative data, making it possible to compare the MIC values reported by different researchers and at different times. The MIC_{50} and MIC_{90} of cloxacillin was determined as the lowest concentration of the antibiotic that was found to inhibit bacterial growth Microdilution method used according to guidelines (NCCLS 2000/CLSI 2009; NCCLS/CLSI-M07-A5/M07-A8). No breakpoints (CLSI, EUCAST) were found. The MIC was described and correlated with the concentration of cloxacillin at the different times at the dry period (every week analysis, data obtained from the internal study Pfizer 2369). The analysis considers the data of commercial formulations in Germany (mostly 1 g cloxacillin per syringe) and their formulation (smaller cloxacilin particle size ('dynomilling') or not).

Results

200 samples from 62 farms (mean 3,22 samples per farm, min 1, max 27) were analyzed. The MIC values *S.uberis*/Cloxacillin were between 0,125 µg/mL (1% of the samples) and 64 µg/mL (1% of the samples), median value was 2 µg/mL. MIC_{50} and MIC_{75} were 2 µg/mL and MIC_{90} was 4 µg/mL. The MIC related are according with De Jong et al. (2018) and Bolte et al. (2020).

The effectiveness of antimicrobial therapy depends on several factors like formulation and dose. The mean concentration of 1g cloxacillin post administration is related as following: 1st week: 142 µg/mL; 2nd week 92 µg/mL; 3rd week 89 µg/mL; 4th week 35 µg/mL; 5th week 8 µg/mL and 6h week 3 µg/mL using dynomilling and ”cloxacillin without dynomilling” related the concentrations: 1st week: 161 µg/mL; 2nd week 29 µg/mL; 3rd week 10 µg/mL; 4th week 6 µg/mL; 5th week 0.3 µg/mL and 5th week 0.09 µg/mL (data from the internal study Pfizer 2369).

Conclusion

The pharmacokinetic behavior of cloxacillin with very high concentrations following intramammary infusion at drying off with 1 g, specially using dynomilling and the MIC values obtained shows that the use of cloxacillin provides sufficient respond to *S. uberis* across the dry period.

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1643 - Prevalence of Mycoplasma bovis in German cattle herds

Author: Torsten Steppin, Andrea Lidner, Ingrid Lorenz, Ulrich Löschner, Ansgar Busch, Thomas Breuer, Marcus Klawitter, Luis Leon

Objectives

Mycoplasma bovis (*M. bovis*) is a common pathogen in cattle. Infections with *M. bovis* induce several clinical signs in cattle of all ages, breeds and farming types. Most common are respiratory diseases in calves, as well as mastitis and arthritis in older cattle. PCR is common to detect *M. bovis* in samples from the respiratory system (deep nasal swabs, bronchoalveolar samples (BAL)). The detection of antibodies in blood or milk is possible with ELISA. To get an overview of the dairy herds in Germany, a collection of bulk milk samples in more than 400 dairy herds was performed. Additionally, samples from acute affected calves were taken by BAL, deep nasal swabs or at necropsy.

Material and methods

Bulk milk samples were collected randomly from vets at dairies (Holstein Friesian and Simmental herds) in relevant dairy areas all over Germany. The samples were used for detection of *M. bovis* antibodies with the test ID Screen® Mycoplasma bovis Indirect ELISA (ID vet, France) at the laboratory of the Bavarian Animal Health Services. Samples from acutely diseased cattle were taken and examined by the laboratory Biocheck (Germany) for respiratory pathogens using the VetMAX™ Ruminant Respiratory Screening PCR Kit (Thermo Fisher Scientific Inc, USA).

Results

Bulk milk samples from over 400 dairy herds were collected and analyzed. Over 25% of the samples were positive for *M. bovis* antibodies. From January 2022 till end of 2023 about 200 calves from 41 farms, most of them dairy farms, were sampled by BAL and examined by PCR after pooling the samples from each farm. About 30% of the pooled samples were positive for *M. bovis*. The ELISA results show that antibodies against *M. bovis* can be detected across all German areas.

Conclusions

The results show a higher prevalence of *M. bovis* antibodies in German dairy herds compared to former investigations in specific German areas (Lorenz, 2023). The new data is in line with reports from other European countries (Gille et al. 2018; McAloon et al. 2022). *M. bovis* is a major pathogen in German cattle production.

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1649 - Detection and quantification of the E6 oncogene of bovine papillomavirus types 2 and 13 from urinary bladder lesions of beef and dairy cattle.

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Objectives

Bovine papillomavirus types 2 (BPV2) and 13 (BPV13) can induce tumors in both the cutaneous and mucosal epithelia of cattle, leading to distressing animal diseases, and considerable economic losses. These viral types are also associated with malignant lesions in the urinary bladders, known as bovine enzootic hematuria. This clinical syndrome causes intermittent hematuria, anemia, progressive emaciation, and death in affected animals. Carcinogenesis observed in the urinary bladder has been associated with synergism between BPV infection and chronic intoxication by bracken fern (*Pteridium aquilinum*) ingestion, which contains mutagenic and carcinogenic principles as quercetin and ptaquiloside. These compounds trigger viral gene expression that results in cell transformation. Along with the E5 viral protein, which is believed to be the major oncoprotein of BPVs in the *Deltapapillomavirus* genus, the oncoprotein encoded by the E6 gene has an important role in cell proliferation and might be related to cancer initiation and promotion. The aim of this study was to standartize the SYBR Green-based quantitative PCR for the detection and quantification of the BPVs 2 and 13 E6 oncogenes in urinary bladder samples from cattle.

Material and methods

Twenty-four urinary bladders from cattle displaying tumors (*n*=12) and normal bladder mucosa (*n*=12) were collected from adult bovines from two different farms in a slaughterhouse in southern Brazil. Urinary bladder carcinomas were confirmed by histopathology. Two sets of specific primers BPV-2 and 13 specifics were designed and were able to amplify approximately 120 bp of the E6 ORF. Six 10-fold serial dilutions of BPVs 2 and 13 plasmids were used to generate a standard curve by plotting the logarithm of the plasmid copy number against the measured Cq values. The BPV2 Cq values ranged from 15.99 to 32.56 cycles with a linear correlation (R2) of 0.999 (slope = −3.488) between the Cq value and the logarithm of the BPV2 copy number. The BPV13 Cq values ranged from 13.03 to 30.51 cycles with a linear correlation (R2) of 0.998 (slope = −3.698) between the Cq value and the logarithm of the BPV13 copy number. The efficiency of the two qPCR assays was 98.2 and 96.7%, respectively. The limit of detection of the qPCR was evaluated by testing six 10-fold serial dilutions of the DNA standards, which contained 1.14×10^6 down to 1.14×10^1 DNA copies/µL of BPV2 and 1.54×10^6 down to 1.54×10^1 DNA copies/µL of BPV13.

Results

The qPCR identified 11.4 and 15.4 copies of BPV2 and 13 DNA, respectively. The BPV2 qPCR intra-assay coefficients of variation (CVs) ranged from 0.065 to 1.077%, while the interassay CVs ranged from 3.81 to 9.99%. The BPV13 qPCR intra-assay CVs ranged from 0.028 to 2.77%, and the interassay CVs ranged from 4.04 to 7.6%. Of the 12 urinary bladders with tumors tested by quantitative PCR, six presented BPV2 DNA concentrations ranging from 1.05×10^4 to 9.53×10^3 copies/µL, while two had BPV13 DNA amplified at concentrations of 1.30×10^4 to 1.23×10^4 copies/µL. The healthy bladder mucosa samples were negative for both bovine papillomaviruses.

Conclusions

Once the results were confirmed by conventional PCR and direct sequencing, the qPCR assay developed in this study was shown to be a sensitive and specific tool for detecting and quantifying the E6 ORF of BPVs 2 and 13. This approach represents the first qPCR technique for the detection and quantification of BPV13 DNA. The use of this molecular tool will aid in testing diverse clinical samples from a high number of animals suspected of suffering from infections with these viral types, at a lower cost and without the need for direct sequencing to confirm the BPV type identities. Despite the common occurrence of chronic BEH in cattle from several geographic regions around the world that are known for being endemic with this disease, the identification of an association of BPV13 DNA with clinical specimens collected from the affected cattle is still scarce. Our findings of identification of BPVs 2 and 13 DNA in urothelial tumors from cattle suffering from BEH agree with data from previous studies, representing the first detection of BPV13 DNA in malignant bladder lesions of cattle from Brazil.

Financial support: National Institute of Science and Technology for Dairy Production Chain (INCT–LEITE), CNPq, CAPES, and Fundação Araucária (FAP/PR).

1658 - Methicillin resistance and biofilm production in Staphylococcus spp by multiplex PCR isolated from bovine mastitis

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Objectives

Biofilm production may be mediated by the ica operon which includes the icaA, icaB, icaC and icaD genes. The icaA and icaD genes together regulate the phenotypic expression of the biofilm forming polysaccharide. Treatment of bovine mastitis by Staphylococcus aureus is complicated due to resistance of bovine mastitis by Staphylococcus aureus is complicated due to resistance to B-lactams, persistance of infection in the mammary cland due to the production of biofilm which protects microorganisms from the action of antimicrobials. The objective of the work was to desgns a multiplex PCR by means of which the biofilm production capacity, intrinsic resistance to methicillin and the expression of resistance to methicillin in Staphylococcus spp

Material and methods

Milk samples of cows were isolated, identified by API Staph. DNA extraction was performed from colonies using CTAB, standardized to 20 ng/ul. The reaction was done at a volume of 25.5 ul of which 10.5 ul corresponded to GoTaq Green Master Mix from Promega, 7 ul corresponded to nucleotide-free water and 1 ul of each pair of oligonucleotidos for each each gene with a concentration of 20 pmol/ul (icaD, mecA and femA). The program used consisted of initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30 seg, alignment at 55.5 °C for 30 seg and extension at 72°C for 30 seg and extension at 72°C for 30 seg; finally and extension at 72°C for 3 min. The PCR products were evaluated by electrophoresis in a 1.5% agarose gel using ethidium bromide as an intercalant. The electroforesis was run for 90 min at 80 V. Finally, the gel was visualized in a photodocumentator with UV light.

Results

54 Staphylococcus were isolated, of which 22 S. aureus were identified, of these only 9% presented the mecA gene, however 81% presented the femA gene indicating the ability to express resistance to methicillin and 27.2% were positive for the gene icaD showing the ability to produce biofilm throug the mechanism regulated by the ica operon. In the case of coagulase negative Staphylococcus, 9% of the strains presented the mecA gene, 28% the fem A gene and 6% presented the icaD gene

Conclusions

The femA gene was not found exclusively in S.aureus strains, it was also found in coagulase negative Staphylococcus. The percentage of strains positive for the mecA gene is low compared to that reported in strains of human origen, however they are similar to that reported in strains isolated from milk. The mecaA gene is found in a smaller proportion that the femA gene.

1668 - Outbreak of septicemic Salmonellosis in dairy calves

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Gianneechini, Marcos Schanzembach , Carolina Matto

Objectives

The aim of this work is to describe an outbreak of Salmonellosis in calves at the end of the calf-rearing period on a dairy farm of Paysandú, Uruguay.

Material and methods

The outbreak initiated 7 days after the milk feeding finished. In that moment, calves consumed 1.5 kg of concentrate (dry matter) per animal/per day and Moha (*Setaria italica*) hay *ad libitum*. Two calves, of 26 animals (7.7%), presented anorexia, recumbency and markedly depression. One of them died the same day (id 2210, 78 days of age). The other calf (id 2213, 71 days of age) showed apathy, rectal temperature of 40.3°C, cardiac rate of 136 beats/min, respiratory frequency of 48/min, oral and ocular mucosa pale, diarrhea (liquid yellow feces), middle dehydration and died at 24h. Necropsy of both calves was performed for diagnosis.

Results

Both animals presented similar gross lesions. The mucosa oral and ocular were pale-icteric, as well as the serosa of all abdominal and thoracic viscera. In all the serous membranes there was also multifocal hemorrhages. Both animals had marked hepatomegaly and splenomegaly. The liver parenchyma had a diffuse orange color. There was no hemoglobinuria. There was adenomegaly in mesenteric lymph nodes. In the small intestine, the mucosa had hemorrhagic. The lungs were edematous and hemorrhagic. Samples of viscera were fixed in buffered formalin at 10% for histopathological study and samples of spleen, liver, bile, kidney and lung were conserved at 4°C for bacteriological studies. In all samples cultivated, it was isolated *Salmonella dublin*. At histopathology, the main lesions were in the liver, characterized by a multifocal to coalescent lymphosuppurative hepatitis with bile retention in sinusoids and bile ducts. The gall bladder had transmural edema of the wall and multifocal areas of necrosis in mucosa. In kidney were found moderate interstitial infiltration of lymphocytes. In spleen was found neutrophilic splenitis with presence of intralesional gram-negative bacteria. The mesenteric lymph nodes had multifocal areas of necrosis in cortex and neutrophilic infiltrate. The small intestine had necrolymphosuppurative enteritis, multifocal and severe whereas the large intestine had mild lympho colitis and infiltration of the lamina propria of the mucosa by lymphocytes, diffuse, moderate. In lung there was lesions of septicemia characterized by distention of the alveolar septa by lymphocytes and macrophages, and intraalveolar proteinaceous substances.

Conclusions

The gross and microscopic lesions mentioned above are characteristics of a septicemic condition caused by *Salmonella dublin* infection. The morbidity, mortality and lethality rates were of 7%, 7%, and 100%. The restriction in feeding due to the suspension of the milk diet, the change in the kind of diet and the insufficient amount of ration, undoubtedly generated stress in the animals and was a determining factor for the manifestation of the disease. It is highlighted that in this farm there has been no diagnosis of Salmonellosis in calves for more than 20 years. *S. dublin* is an infection transmitted from animal to animal and animals are often asymptomatic carriers. In this outbreak the change in management produced the onset of clinical salmonellosis in

animals that probably were infection preexists. *Salmonella dublin* generally affects calves over 2 months of age, the same situation that occurred in this outbreak.

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1669 - Antimicrobial resistance and biofilm production in bacteria causing caprine mastitis

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Objectives

Mastitis in goats can occur chronically or severely, caused by different causes: such as the presence of pathogenic microorganisms in the mammary gland or traume. It is characterized by physical and chemical changes in milk. Althought mastitis is invariably associated with bacterial invasion, predisposing factors such as age, stage of lactation, number of partities are of great importance. Mastitis is less common in dairy goats and the information available on the different types of etiological agents that cause the disease in goats is less than that available for cattle. The objectives were the identification of the etiological agents causing subclinical mastitis and determination of antimicrobial susceptibility and determination of biofilm production for effective treatment

Material and methods

Five milk production units in the state of Coahuila and two production units in San Luis Potosí were visited; During the milking process, the California Test (CMT) was performed on 111 goats. For bacteriological diagnosis, samples were plated on Blood agar and MacConkey agar for bacterial identification and Sabouraud dextrose agar for the identification of fungi and yeasts. Primary and secondary biochemical tests were carried out to carry out bacterial identification, in addition API Staph and API E identification microsystems were used.Antibiotic susceptibility testing:The Kirby-Bauer method was used on Müller-Hinton (MH) agar, a bacterial strain standardized to 0.5 on the Mcfarland scale was inoculated and commercial multidiscs (Gram positive or negative) were placed, incubated at a temperature of 37°C. for 18 to 24 h, subsequently the inhibition zones were measured in mm, to classify them as sensitive, intermediate or resistant.Biofilm production was determined in Congo red agar; brain heart infusion agar supplemented with 3.6% sucrose and 0.08% Congo red dye was used, which were simultaneously sterilized in an autoclave. The strains were seeded and incubated for 24 h at 37°C, then incubated again for a new period of 24 h. The Cowan strain of Stpahylococcus aureus was used as a positive control. The observation of black colonies with a crystalline appearance was considered positive, red colonies in Congo red agar were considered negative for biofilm production.

Results

Of the 111 milk samples analyzed, 11 % were bacteriologically negative. Among the 89% of positive samples, different bacterial species causing mastitis were found, S. xylosus 9/87 (10.34%), S. lentus 8/87 (9.19%), S. hominis 5/87 (5.74%), S. haemolyticus 3/87 (3.44%), S. auricularis 3/87 (3.44%), S. sciuri 1/87 (1.14%), S. warneri 1/87 (1.14%), S. epidermidis 1/87 (1.14%), S. saprophyticus 1/87 (1.14%). The bacterial isolation of coagulase-positive Staphylococcus was: S. intermedius 2/87 (2.29%). Other bacterial genera were isolated such as: Bacillus panthotenticus 3/87 (3.44%), Bacillus cereus 1/87 (1.14%) and Bacillus coagulans, Micrococcus kristinae 1/87 (1.14%). The genus Streptococcus was infrequently isolated: Streptococcus agalactiae 1/87 (1.14%), Streptococcus arginosus 1/87 (1.14%) and Streptococcus equisimilis /87 (1.14%).In the

case of Gram negative bacteria, isolates were obtained: Mannheimia haemolytica 10/87 (11.49%), Aeromona spp 4/87 (4.59%), Arcobacter spp 4/87 (4.59%), Pasteurella multocida 1/87 (1.14%), Klebsiella pneumoniae 1/87 (1.14%), Alcaligenes fecalis 1/87 (1.14%). 100% of the bacteria were sensitive to the antibiotics Levofloxacin and Trimethoprim Sulfamethoxazole, and 14.28% resistant to Cefotaxime, Cefuroxime and Cefepine, using the Kirby Bauer technique. Biofilm production was low in the isolated bacteria, 18%, and was detected in Mannhaemia haemolytica and coagulase-negative Staphylococcus

Conclusions

With the above it can be seen that there is a wide variety of species that cause caprine mastitis, however, its presentation will depend on a wide variety of factors related not only to the animal but also to its environment. Likewise, it can be seen that indiscriminate use of antibiotics without knowledge of mastitis severity levels and culture-based therapy causes some resistance. It is concluded that the most frequent microorganism in caprine subclinical mastitis in this study was S. chromogenes currently called Mammaliicoccus and another microorganism that was found more frequently was Mannheimia haemolytica. Low antimicrobial resistance was found as well as low biofilm production.

1677 - Detection of the BVDV in bovine aborted fetuses with use of cavity fluids and combined techniques

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Objectives

The challenge of the livestock industry is to increase beef and dairy system production. A common feature in both systems is abortion and perinatal mortality. The definitive etiological diagnosis in fetuses from possible abortion due to an infectious cause is essential to improve disease management and prevention and to increase the efficiency of production systems. Previous studies in Argentina have demonstrated that nearly 50% of abortions are associated with bacterial, protozoal, or viral diseases. However, the etiological diagnosis efficacy of bovine abortion is still below 50%. Establishing BVDV as the etiological agent of abortion or neonatal death is often a difficult task. The present work aimed to improve the efficiency of the BVDV diagnostic in fetuses from cavity fluid samples.

Material and methods

Eighty-one samples of bovine fetuses' cavity fluids (CF) were selected for this analysis. All cases were submitted for postmortem examination by the Specialized Veterinary Diagnostic Service (INTA EEA Balcarce) and the necropsies were performed according to the guidelines proposed by [1]. These samples were classified into four groups according to the diagnosis obtained in each case. Group 1 (n=9), aborted fetuses with BVDV isolation (BVDV + VI); group 2 (n=33), aborted fetuses with confirmation of infection with the protozoan *Neospora caninum* (*Nc* + PCR); group 3 (n=27), aborted fetuses with histopathological lesions without isolation of an infectious agent (probable infectious abortion, PIA) and group 4 (n=12), aborted fetuses without histopathological lesions or abortigenic agents (non-infectious abortion, NIA).

The first step was clarifying the CF, and centrifugation for 10 minutes at 19,000 g. Then RNA extraction was carried out using the commercial TIANamp® Virus RNA kit, following the manufacturer's instructions. All RNAs were analyzed by RT-PCR, according to [2] for detection of the 5'UTR segment. The positive samples of this technique were analyzed by nested RT-PCR according to [3] to differentiate BVDV-1 from BVDV-2, for detection of the NS5B gene. In addition, all samples were analyzed for detection of RNA of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH), for quality control of the extracted RNA. All samples were analyzed using the ELISA IDEXX® Bovine Viral Diarrhea Virus (BVDV) Antibody Test Kit, following the manufacturer's instructions. In addition, all samples were processed for viral neutralization (VI) for the detection of neutralizing antibodies against BVDV.

Results

In group 1, 5'UTR segment was detected in all samples (9/9, 100%). Two of the nine samples were detected for genotype 2 and one for genotype 1. In group 2, two of the 31 samples (2/31, 6,45%) were positive for the detection of the 5'UTR segment of BVDV. These two samples were analyzed to differentiate the genotypes and only one could be classified as BVDV-1. In group 3, four of the 27 samples (4/27, 14.81%) were positive for the detection of the 5'UTR segment of BVDV. These four samples were analyzed by

nested RT-PCR and only two could be classified as BVDV-1. In group 4 one sample (1/12, 8.33%) resulted positive for the PCR 5'UTR segment which could not be classified into BVDV-1 or BVDV-2. In all the samples of groups 2, 3, and 4, a 103 bp segment corresponding to the GAPDH endogenous gene was verified confirming the quality of the RNA. In group 2, five of the 31 samples (5/31, 16.13%), in group 3, four of the 27 samples (4/27, 14.81%) and group 4 one sample (1/12, 8.33%) were positive for the detection of sAb against BVDV.

Conclusions

The increase in sensitivity is possibly linked to viral viability since VI only detects viable viruses and molecular techniques detect both viable and non-viable viruses. The autolysis process is a factor dependent on time and the way the specimens are preserved, which directly impacts the efficiency rates of abortion diagnosis. The detection of fetal antibodies indicates the simple exposure of the fetus to the agent, which is not sufficient to establish that BVDV is the cause of the abortion. In conclusion, the CF turned out to be a diagnostic possibility, since it was shown to contain viral genetic material in a quality RNA for the reaction, being representative of the fetus. Although diagnostic techniques are becoming more precise every day, the diagnosis of abortion continues to represent a challenge for professionals dedicated to this task.

1681 - Assessing the nasopharingeal microbiome following BVDV2+BHV1 challenge in dairy calves submitted to different vaccination protocols in combination or not with injectable trace minerals.

Author: Roberto Palomares, Alejandro Hoyos-Jaramillo, Joao Bittar, Erika Gonzalez, Adriana Rodriguez, Jose Urdaneta, Jeferson Lourenco

Objectives

The upper respiratory tract (URT) microbiome includes opportunistic bacteria that exacerbate and cause respiratory disease when the respiratory mucosa and the immune system are disrupted. Pathogens such as *Bovine viral diarrhea virus 2* (BVDV2) and *Bovine herpes virus 1* (BHV1) can cause immunosuppression and damage of the URT mucosa favoring secondary bacterial infection and clinical disease. The use of injectable trace minerals (ITM) concurrent with vaccination improves the immune response and protection elicited against respiratory viruses in calves. However, little is known regarding the effects of vaccination and ITM administration on the URT microbiota.

The objective of this study was to determine the richness, diversity and relative abundance of the nasopharyngeal bacterial phyla and genera following BVDV2 + BHV1 challenge among calves submitted to modified-live virus (MLV) booster vaccination (either intranasal or subcutaneous) concurrent with injectable trace minerals (ITM) or saline.

Material and methods

Forty-eight dairy calves (1 mo) were prime vaccinated with a MLV IN vaccine containing BHV1, *Bovine respiratory syncytial virus* (BRSV), *Bovine parainfluenza 3 virus* BPI3V (Inforce 3®), and randomly assigned to subcutaneous (SC) administration of ITM (ITM, n=24; Multimin®90 containing Se, Cu, Zn & Mn) or saline (Sal, n=24). Ten weeks later, calves received a second dose of ITM, or saline, according to previous groups and were randomly assigned to receive the same IN vaccine [ITM-IN (n = 12), SAL-IN (n = 12)] or a SC MLV vaccine containing BHV1, BRSV, BPI3V, BVDV1 & 2 [ITM-SC (n = 12), SAL-SC (n = 12)]. Additionally, 12 calves did not receive vaccine or treatment and served as a control group (UNVAC, n = 12). Seven weeks after booster vaccination, all calves were intranasally inoculated with BVDV2 (5 x10^5 Tissue culture infecting dose 50%; $TCID_{50}$); and seven days later with BHV1 (8 x10^6 $TCID_{50}$). From each calf, nasal swab samples were collected 49 days after booster vaccination (day 0, baseline before BVDV2 challenge), 5 days after BVDV2 challenge (day 5) and 5 days after BHV1 challenge (day 12). Nasal swab samples were frozen at -80 ºC until submission to Fera Biologicals and Diagnostics Corp for bacterial metagenomic analysis to characterize the URT microbiome by using high-throughput sequencing of the 16S rRNA. All statistical analyses were performed using the Statistical Analysis System (SAS®). Normality and constant variance assumptions were assessed by using Shapiro Wilk's and Levene's tests, respectively. Relative abundance of bacterial phyla and genera were compared among treatment groups by using a repeated measures analysis model (Proc-GLIMMIX), using calf ID as a random effect, and the interaction of treatment groups and virus challenge as fixed effects. The results were considered significant when $p \leq 0.05$, and a statistical tendency was considered when $0.05 < p \leq 0.10$.

Results

Six major phyla were identified with a relative abundance >1% (*Protobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Tenericutes* and *Fusobacteria*). Relative abundance of bacteria Phyla changed following BVDV2 + BHV1 challenge episodes (*Protobacteria P<0.0001; Firmicutes P<0.0001; Bacteroidetes P<0.006; Actinobacteria P< 0.006; Fusobacteria P= 0.03; Tenericutes P= 0.02*). Administration of trace minerals and the route of vaccination were not associated with changes in relative abundance of bacteria Phyla (P > 0.05). Relative abundance of bacteria commonly involved in BRD (*Mannheimia, Pasteurella*, and *Mycoplasma*) was not influenced by trace mineral supplementation, booster vaccination (49 days before microbiome assessment) or BVDV2 + BHV1 challenge. Virus challenge was associated with changes (P< 0.001) in relative abundance of relevant bacteria genera (*Ruminococcus, Prevotella, Pseudomonas, Blautia, Acinetobacter, Psycrobacter, Corynebacterium, Bacteroides, and Moraxella*).

Conclusions

In conclusion, the route of booster vaccination and ITM administration did not affect the nasopharyngeal microbiome seven weeks following vaccination and treatment. Moreover, BVDV2 + BHV1 challenge (regardless of the vaccination and trace mineral status) altered the relative abundance of bacteria phyla and genera in the studied dairy calves.

1692 - SEROPREVALENCE OF BOVINE ENZOOTIC LEUKOSIS IN CATTLE HERDS IN MEXICO

Author: Ana Delia Rodríguez Cortez, Lucía E. Rangel, Arantzatzu Lassala, David Martínez-Herrera, Rogelio Alonso, Carlos G. Gutiérrez

Objectives

To determine the apparent seroprevalence of Bovine Enzootic Leukosis (BEL) across diverse Mexican regions and to understand the associated risk factors influencing its spread among cattle populations.

Material and methods

A total of 2686 serum samples from clinically healthy, unvaccinated, grazing cows over 2 years old were collected from 430 municipalities across 30 states of Mexico. The country was divided into 5 regions (North, Gulf, Center, Peninsula, and Pacific) based on geographical, agroecological, and management similarities. Seropositivity was assessed using a Luminex® immunoassay targeting the gp51 antigen of the bovine leukemia virus, validated with 96.43% sensitivity, 96.8% specificity, AUC= 0.981 (CI 95% 0.95-1.0), LR(+) 29.89, and CV R (4.93%) r (5.1%). Logistic regression was employed to calculate the prevalence, and the relative risk (RR) was assessed for environmental factors such as temperature, humidity, climate, and production purpose, alongside animal age, type of service, seasonality of mating, and grazing type.

Results

The study found high general seropositivity across Mexican regions: 42.3% in the North, 53.6% in the Pacific, 45.2% in the Center, 43.3% in the Gulf, and 32% in the Peninsula. These seropositivity levels indicate mesoendemic to hyperendemic levels. No significant differences were found between regions. Humidity significantly affected BEL prevalence ($p \leq 0.05$) with prevalences and relative risk in subhumid environments higher than in very arid environments (Pr= 51%; RR=1.11 vs. Pr= 27%; RR=0.57). Similarly, the type of breeding ($p \leq 0.05$) affected prevalence, with continuous mating systems showing lower prevalence and RR (Pr= 47%; RR=1.68) compared to seasonal breeding (Pr= 28%; RR=0.59).

Conclusions

The research indicates widespread prevalence of BEL in Mexican cattle, with specific environmental and management factors significantly impacting seropositivity rates. These findings underscore the need for targeted surveillance and control measures, especially considering the high endemic levels and the lack of effective vaccines. Further, the presence of antibodies in an unvaccinated population indicates exposure to the field virus. However, the control is difficult due to late (1 to 5 years) and low incidence (40%) of clinical manifestations, and the nature of the retrovirus leads to persistent infections and the possibility of reactivation of the disease. Hence, actions should be directed to the detection of seropositive animal, and the determination of the proviral loads to inform the segregation and culling of highly infectious cows. This study contributes to a broader understanding of BEL's epidemiology in Mexico and can guide future health and trade policies.

1693 - RESPIRATORY RISKS: TRACING PI3 AND BRSV IN MEXICAN CATTLE

Author: Ana Delia Rodríguez Cortez, Oscar Rico-Chávez, Alejandro Zaldivar-Gómez, Lucía E. Rangel, Arantzatzu Lassala, Rogelio Alonso, Carlos G. Gutiérrez

Objectives
To determine the apparent seroprevalence of infections caused by Parainfluenza 3 (PI3) and Bovine Respiratory Syncytial Virus (BRSV) in Mexico and understand the associated risk factors influencing its spread among cattle populations.
Material and methods
A national sampling was conducted, calculating a minimum sample size of 2,686 sera from 30 entities of the Mexican republic. Samples were taken from grazing animals, clinically healthy, unvaccinated, and older than 2 years, originating from 430 municipalities. The Mexican territory was zoned into 5 regions (North, Gulf, Center, Peninsula, and Pacific) that share geographical proximity, agroecological conditions, and similar management practices. Samples were diagnosed for seropositivity to the HN protein of the PI3 virus and whole BRSV on a Luminex® immunoassay platform standardized and validated by our laboratory with 100% sensitivity and 93.1% specificity for PI3, and 100% sensitivity and 96.67% specificity for BRSV. Prevalences were calculated by logistic regression, and relative risks (RR) were calculated for environmental factors such as temperature, humidity, climate, as well as zootechnical purpose, age of the animals, type of service, breeding seasonality, and type of grazing.
Results
The seropositivity found in each region was 47.8% (PI3) and 49.3% (BRSV) for the North; 51.2% (PI3) and 58.8% (BRSV) for the Pacific; 44.2% (PI3) and 51.2% (BRSV) for the Center; 52.9% (PI3) and 51.2% (BRSV) for the Gulf; and 40.1% (PI3) and 47.7% (BRSV) for the Peninsula, with no significant statistical differences between the regions of Mexico. Humidity and type of mating were significantly different risk factors ($p \leq 0.05$), with higher prevalences and RR in subhumid environments and continuous mating systems. Prevalences of up to 55% and a Relative Risk (RR) of 1.15 were observed in humid environments vs. prevalence of 29% and RR of 0.59 in very arid environments for PI3 infection. For BRSV a prevalence of 60% and RR of 1.16 were noted in subhumid environments, and of 28% and RR of 0.52 in very arid environments. Continuous mating resulted in prevalences of 50% and RR of 1.67 for PI3 and 55% with RR of 1.45 for BRSV, while seasonal mating showed a prevalence of 30% and RR of 0.60 for PI3 and 38% RR of 0.69 for BRSV.
Conclusions
The seroprevalence found for PI3 and BRSV across the different regions of Mexico are within meso- to hyperendemic levels, considered high endemicity levels indicative of pathogen exposure. Despite both respiratory infections being considered high morbidity and low mortality, their economic impact due to maintaining the infection in herds is significant. Therefore, active monitoring activities are crucial to prevent the spread of infections, improve animal welfare, encourage vaccination, and segregate highly positive animals. Additionally, high environmental humidity associated with increased relative risk of seropositivity draws attention to Mexico's coastal areas with higher antibody titers. Continuous breeding significantly increases the risk of seropositivity compared to seasonal breeding, especially given that 97% of herds in Mexico operate

under continuous breeding system. Addressing the endemicity of PI3 and BRSV with effective vaccines, widespread diagnostic techniques, and the segregation of highly positive animals is imperative to prevent the re-emergence of more lethal disease variants, contain its spread, mitigate economic impact, and prevent international trade limitations.

1696 - A blood glucose meter using glucose dehydrogenase accurately measures lactose percentage in bovine milk

Author: Sahar Kandeel, Ameer Megahed, Peter Constable

Objectives
The milk concentration of lactose, a disaccharide of glucose and galactose, decreases in quarters with subclinical mastitis. The [Accu-Chek Aviva®] blood glucose meter measures glucose concentration using glucose dehydrogenase (GDH) in the presence of pyrroloquinoline quinone (PQQ) cofactor. The PQQ-GDH enzymatic process generates a small electrical current that is detected by the glucose meter, with the current being proportional to the glucose concentration. Interestingly, GDH oxidizes a wide range of monosaccharides and disaccharides, including the monosaccharides glucose and galactose as well as the disaccharide lactose. The concentration of lactose in bovine milk (≈136mM) is much greater than that of glucose (≈0.1mM) or galactose (≈0.2mM). We therefore hypothesized that a glucose meter using PQQ-GDH methodology may provide an accurate method to measure lactose percentage in bovine milk and consequently detect quarters with subclinical mastitis.
Material and methods
Lactose, glucose, and galactose were added to distilled water, milk protein solutions, and bulk tank milk at 37°C to provide 0, 1, 2, 3, 4, 5, 6, and 7% test solutions. In addition, 412 quarter cisternal milk samples were obtained from 106 healthy dairy cows without clinical signs of mastitis at 4-7 days of lactation. A test strip was inserted into the glucose meter and a drop of the test sample was applied to the end of the test strip. The test result was displayed on the screen within 5 seconds. Milk somatic cell count (SCC) was determined using a [Delaval®] cell counter.
Results
There was an excellent positive linear correlation ($r>0.98$) between the meter displayed value in mg/dL and lactose ($P<0.001$), glucose, and galactose percentages in distilled water, milk protein solutions, and bulk tank milk. Compared to changes in milk glucose concentration, the meter was less responsive to changes in galactose and lactose concentrations based on relative slope values to glucose of 53% and 13%, respectively. Nevertheless, the meter provided an accurate estimate of milk lactose percentage, as the sum of glucose and galactose concentrations in milk measured by the meter was <5% of the measured lactose concentration. For bovine milk, we determined that: lactose %= 0.0092×[meter value]-1.3. Segmented linear regression indicated that mean lactose percentage (typically 4.7% in healthy quarters) decreased linearly ($P<0.001$) with increased $\log_{10}$(SCC) in milk samples when the meter value was <654 mg/dL.
Conclusions
The [Accu-Chek Aviva®] glucose meter provides an accurate, rapid, practical, and low-cost method to measure milk lactose percentage. Because of its ability to quantify milk lactose percentage, the meter can be used to detect the presence of subclinical mastitis, thereby offering a practical cow-side method to identify quarters for selective dry cow treatment and to monitor the response of mastitic quarters to treatment.

1699 - Assessing IBR seroprevalence and charting the path to mitigation.

Author: Ana Delia Rodríguez Cortez, Lucía E. Rangel, Arantzatzu Lassala, Rogelio Alonso, Carlos G. Gutiérrez

Objectives

To determine the apparent seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in Mexico and its association with risk factors, we conducted an immunoassay on the Luminex® platform. This study aimed to understand the prevalence of IBR among cattle populations and the environmental and management factors influencing its spread.

Material and methods

Two thousand six hundred and eighty six sera samples from grazing cows that were clinically healthy, unvaccinated, and older than 2 years, from 430 municipalities were used. For the seroepidemiological study, Mexico was divided into 5 regions (North, Gulf, Center, Peninsula, and Pacific) sharing geographical proximity, agroecological conditions, and similar management practices. The samples were diagnosed for seropositivity to the IBR virus in a Luminex® assay previously standardized and validated with 100% sensitivity, 98.8% specificity, AUC= 0.999 (CI 95% 0.99-1.0); LR(+) 83.0, and CV R (10.84%) r (6.39%). The prevalence and relative risks (RR) were determined for environmental factors such as temperature, humidity, and climate, as well as production purpose, age of the animals, type of mating, breeding seasonality, and type of grazing.

Results

The seropositivity found in each region was 36.1% for the North; 37.4% for the Pacific; 31.1% for the Center; 34.6% for the Gulf, and 30.2% for the Peninsula, without significant statistical differences between regions of Mexico. Analysis of associated risk factors showed that only humidity and type of mating were significantly different ($p \leq 0.05$), with prevalences of 39% and RR 1.16 in subhumid environments vs. 20% and RR 0.57 in very arid environments. Continuous mating had a prevalence of 35% and RR of 1.96, while seasonal mating had a prevalence of 18% and RR of 0.51. Although the levels of endemicity are moderate, the persistence in the national herd represents a risk for international trade, as several regions and countries are considered IBR-free zones with restricted trade from prevalent areas.

Conclusions

The seroprevalence of IBR in Mexico's cattle populations is at mesoendemic levels, indicating widespread pathogen exposure. Both high environmental humidity and continuous breeding are associated with increased risks of seropositivity. Given that 95% of IBR infections are subclinical and the infection can remain latent with the potential for reactivation, addressing IBR endemicity is crucial. This includes using effective marker vaccines (DIVA) to protect fetuses, establishing mass diagnostic techniques for segregating positive animals who remain as carriers with a high risk of infection reactivation to prevent dissemination, and mitigating the economic consequences of the infection caused by abortions.

1706 - Farmers' knowledge of Johne's disease/paratuberculosis and opinions of the Irish Johne's Control Programme

Author: Louise Horan, John Mee F, Niamh Field, Siobhan Walsh, Ainhoa Valldecabres

Objectives
Johne's disease is an infectious, enteric, wasting disease present in the Irish cattle population. A voluntary control programme, the Irish Johne's Control Programme (IJCP) was set up in 2017. The objective of this study was to assess Irish beef and dairy farmers' knowledge about Johne's disease, the Johne's disease management practices they implemented and their opinions about the IJCP.
Material and methods
A questionnaire, which consisted of 14 questions divided into three sections, gathered herd and participant demographics and assessed general Johne's disease knowledge and IJCP opinions. The questionnaire was distributed in digital format via social media and via email to dairy and beef farmers in two farm discussion groups.
Results
In total, 126 respondents' answers were suitable for analysis; these responses came from mostly young farmers (18-25 years old). Most respondents claimed to know what Johne's disease was (73%; 92/126) and loss of body condition (68%; 78/114) and diarrhea (59%; 67/114) were the two main clinical signs that respondents identified as related to Johne's disease. Twenty-three percent (28/124) of the respondents reported that some of their cattle tested positive in any Johne's disease diagnostic test and among those, the majority were dairy farmers (79%; 22/28). Thirty-eight percent (47/124) of respondents reported that they had implemented management practices to prevent the transmission of Johne's disease within or into their herd. The most commonly reported categories of these type of management practices were management of milk for calf consumption (27%; 12/44) and isolation of Johne's test-positive or newly purchased stock (21%; 9/44). Eighteen percent (22/125) of respondents were, at the time of questionnaire or previously, members of the IJCP. Five of the previous or current members of the IJCP reported having experienced no benefits from partaking in the programme. Of those members who reported a benefit to participation in the IJCP (14/22), the main benefits reported were identification of subclinical cases (29%; 4/14), and no longer feeding Johne's positive cow's milk to calves (21%; 3/14). The main disadvantage of the programme reported by previous and current members at the time of the questionnaire was inaccurate testing methods (50%; 10/20). Of the respondents that had never participated in the IJCP, the main reasons reported for the lack of participation were not being aware of the programme (52%; 53/102) and not having a Johne's disease problem on the farm and therefore not seeing the need to participate in the IJCP (48%; 49/102).
Conclusions
This study suggests that while the majority of these respondents (mostly young farmers) were aware of Johne's disease, participation in the control programme was limited and could benefit from further promotion.

1708 - COMPARATIVE LONGITUDINAL ANALYSIS OF T LYMPHOCYTE SUBPOBLATIONS IN CATTLE VACCINATED WITH DIFFERENT DOSES OF BCG-PHIPPS OR WITH CULTURE FILTRATE PROTEIN EXTRACT OF Mycobacterium bovis, UNDER NATURAL

Author: LAURA JARAMILLO MEZA, FERNANDO DÍAZ OTERO, ANABELLE MANZO SANDOVAL , ROXANA OLGUÍN ALOR, FERNANDO DIOSDADO VARGAS

Objectives

Bovine tuberculosis (bTB) is an infectious disease caused mainly by *Mycobacterium bovis*, produces significant economic losses in the global cattle industry, and it remains a zoonosis of great concern. One of the strategies that have been considered for its control is the application of bacille Calmette-Guérin (BCG) vaccine, so the objective of this study was to perform a comparative analysis and follow-up of the immune response, degree of activation of T lymphocyte subpopulations in calves vaccinated with different doses of BCG-Phipps, or with a culture filtrate protein extract (CFPE) of *M. bovis*.

Material and methods

The study was conducted in a dairy herd of Holstein-Friesian cows with a disease prevalence of 17.7% located in the State of Hidalgo, México, from which calves of 1-4 months were selected to form the different groups of analysis, according to the criteria of negativity to the following immunodiagnostic tests: Single Intradermal Comparative Tuberculin Test (SICTT), gamma-interferon test (BOVIGAM) and an ELISA to evaluate antibody levels. To evaluate the effect of BCG-Phipps vaccine dose or *M. bovis* CFPE on the degree of activation of cell populations, 5 groups of 10 calves each were formed. Groups 1 and 2 were immunized with 300 µg and 600 µg of *M. bovis* AN5 CFPE, respectively; groups 3 and 4 were intradermal immunized with BCG-Phipps vaccine at doses of $1x10^4$ and $1x10^6$ CFU, and group 5 was used as a control. In addition, a group of tuberculin reactors and IGRA-positive calves was included in the analysis, to which $1x10^6$ CFU of BCG-Phipps vaccine was applied, this group was segregated from the TBb-free vaccinated groups. The immune response in the immunized groups was monitored by evaluating IFN-γ production in blood cultures stimulated with *M. bovis* CFPE. Whereas, the response of cell subpopulations, degree of activation and memory marker (CD45) was determined by flow cytometry after *in vitro* antigenic stimulation of peripheral blood mononuclear cell cultures. The data pertaining to IFN-γ production and antibody levels were statistically analyzed by Tukey test and T cell subpopulations were statistically analyzed by Holm-Sidak and Dunn's nonparametric tests, with a significance of $P \leq 0.05$

Results

In the BCG vaccinated groups there was a significant increase in IFN-γ production at 30 days p.v. ($P \leq 0.05$ Tukey test); however, a higher production was observed for the immunized group with a dose of $1x10^6$ CFU of BCG Phipps vaccine. The response in this group was always higher than the other treated and unimmunized control groups throughout the study, with the exception of the response observed for the immunized reactor group, whose production was always high. In general, for population CD4+ statistically significant differences ($P \leq 0.05$) were observed in relation to p.v. time in calves receiving 600µg CFPE, BCG $1x10^4$ and BCG $1x10^6$ CFU. Not so for the CD8+ population, in which no substantial changes were observed throughout the study for

vaccinated and control groups. On the other hand, in all groups a higher percentage of activated helper cells CD4+CD25+ was observed at 30 days p.v. However these increase only were significant in calves receiving 300 µg CFPE and BCG $1x10^4$ ($P \leq 0.05$). In general, progressive increases in this t-cell subpopulation were observed in all groups throughout the study. On the other hand, previous to vaccination, the infected group exhibited clear differences in the percentage of CD4+CD45RO+T cells compared to the percentages determined for this subpopulation in the other groups. In subsequent evaluations, it was observed that Group 2 (600 µg CFPE) showed significant differences ($P \leq 0.05$, Holm-Sidak test) in the subpopulation of memory cooperating T cells (CD4+CD45RO+) 365 and 480 days p.v. with respect to pre-vaccination data and 30 days p.v. Significant differences ($P \leq 0.05$, Dunn's test) were also found in Groups 3, 4, and 5 at 365 and 480 days comparing with data obtained prior to vaccination.

Conclusions

Our results indicate that vaccination with CFPE and *M. bovis* BCG Phipps strain had the following outcomes: a) it stimulated peripheral blood T cell activity, b) it induced a cell-mediated immune response, and c) the development of intradermal test reactivity to BCG vaccine was limited and transient under the doses of BCG vaccine evaluated. This suggests that these vaccines could be successfully applied in bTB control campaigns.

1709 - EVALUATION AND APPLICATION OF TWO MULTIPARAMETRIC PANELS FOR STUDYING LYMPHOCYTE SUBPOPULATIONS BY FLOW CYTOMETRY

Author: LAURA JARAMILLO MEZA, ANABELLE MANZO SANDOVAL , FERNANDO DÍAZ OTERO, ROXANA OLGUÍN ALOR, LUVIA ENID SÁNCHEZ TORRES, FERNANDO DIOSDADO VARGAS

Objectives

Knowledge of the interaction and degree of involvement of immune cells in bovine tuberculosis is crucial, not only for a better understanding of its pathology, but also for the development of basic tools to assist in the eradication of the disease. Therefore, the purpose of this work was to implement and apply two multicolour panels to identify the different T lymphocyte subpopulations, degree of cell activation and immunological memory in calves infected with *Mycobacterium bovis*.

Material and methods

Ten six-month-old Holstein-Friesian calves were selected from a dairy herd located in the state of Hidalgo, Mexico. Five of the calves were positive to the comparative cervical tuberculin test, IFN-γ release assays and ELISA, and five calves were completely negative to the above mentioned diagnostic tests. Peripheral blood samples were collected in vacutainer tubes containing heparin as anticoagulant for separation of mononuclear cells (MNC) by Ficoll-PaqueTM PLUS gradient. Once the MNCs were separated, they were adjusted in RPMI 1640 culture medium supplemented with 10% foetal bovine serum at a concentration of 1x 10^6 cells/ml, and distributed in 48-well plates at a rate of 1.5 ml/well, considering two wells per animal, one of which was stimulated with a protein extract of culture filtrate (EPFC) of *M. bovis* and the other was left as an unstimulated control. Replicates of the cultures were performed considering two incubation periods for three and nine days per event. Plates were incubated at 37 °C in an enriched atmosphere at 5% CO2 and 90% humidity. The three-day cultures were stained with a first panel of monoclonal antibodies (Acm) recognising bovine CD45, CD4, CD8 and CD25 antigens, including an anti-human CD3 Acm cross-reactive with bovine CD3. MNC cultures incubated for nine days were stained with panel 2, which included anti-CD3, CD4, CD8, CD25 and CD45RO Acm. All antibodies were pre-titrated to determine optimal working concentrations. Each panel included an unstained control, compensation controls (CC) and fluorescence minus one (FMO) controls. FMOs were used for the correct quadrant positions of CD25 and CD45RO molecules. The CytoFLEX "S" flow cytometer (Beckman Coulter), equipped with a set of four lasers and filters, was used to collect at least 10,000 events in singlets gate. Data were analysed with FlowJo v.10.8.1 software (Becton Dickinson). For each lymphocyte subset, a specific gating strategy was applied. Evaluation of the multicolour panel included progressive panel construction and fluorescence minus one (FMO). Similarly, the FMO determined for each parameter evaluated showed no decrease in the sensitivity of the conjugates, nor fluorescence signals in the channels for which the conjugate was not added. Results were statistically analysed by Kruskal-Wallis one-way ANOVA, and Mann-Whitney rank analysis of variance was used to assess differences between 3- and 9-day cultures.

Results

Panel 1, which was used in the 3-day cultures, showed a proportion of T lymphocytes (CD3) in the range of 82-90% in cultures from tuberculous and healthy calves in both stimulated and unstimulated cultures with no notable differences between groups. No significant differences were also observed in the CD4+ population for both groups. The number of CD8+ and CD4+CD25+ cells in tuberculous calves was higher after stimulation with CFPE, although not significant. However, there were significant differences in the activated CD8+CD25+ population in MNC cultures from infected calves after antigenic stimulation compared to the unstimulated control ($p < 0.05$). With panel 2, which was used to assess T cell subpopulations in MNC cultures 9 days after antigenic stimulation, no statistical differences in CD3+, CD4+, CD8+, CD8+CD25+ and CD8+CD45RO+ populations were observed between groups. However, there were differences in the percentage of activated CD4+CD25+ cells (37%) in the infected group ($p < 0.001$). The same was true for CD4+CD45RO+ memory cells ($p < 0.01$). CD4+CD45RO+ and CD8+CD45RO+ memory cells were measured after nine days of culture; previous reports indicate differences in this population from day six of stimulation. Tuberculin-positive animals showed a proportion of 83% CD4+CD45RO+ cells and 85% CD8+CD45RO+ cells after stimulation.

Conclusions

Both panels detected differences in T-cell subpopulations between tuberculin positive and negative calves, as well as differences in CD25 and CD45RO expression. Thus, these panels show their usefulness for future research to determine the efficacy of vaccines of interest, or in the course of infections by other pathogens.

1712 - Mycoplasma SPECIES ASSOCIATED WITH RESPIRATORY DISEASE IN TWO DIFFERENT DAIRY CATTLE PRODUCTION SYSTEMS

Author: LAURA JARAMILLO MEZA, Anabelle Manzo Sandoval , Fernando Díaz Otero , Laura Hernández Andrade , Rafael Pérez González, Gustavo Díaz Manríquez

Objectives

Within bovine respiratory disease (BRD), mycoplasmas have a relevant participation; *Mycoplasma bovis* being the species most involved, due to its invasive and pathogenic potential this species can produce systemic and localised infections, which can include mastitis, pneumonia, arthritis, meningitis, keratoconjunctivitis, endometritis and infertility. Under natural conditions this microorganism is frequently isolated with other infectious agents of pneumonic processes, pointing to its synergistic effect in producing lung tissue damage. It is considered the most important microorganism of bovine mycoplasmosis in Europe and North America, and one of the major emerging pathogens threatening bovine production units. *M. dispar* and *M. bovirhinis*, are other mycoplasma species recovered from bovine respiratory diseases. Despite the relevant involvement of mycoplasmas in this type of diseases, they have been neglected, perhaps because the diagnosis of their presence is not made in many of the cases, because of the infrastructure required for their isolation and identification, because the microorganisms are fastidious to their development, The aim of this study was to isolate and identify by PCR the *Mycoplasma* species causing respiratory disease in calves.

Material and methods

Convenience sampling was carried out in two stables, one located in the municipality of Melchor Ocampo, Mexico State, and the other in Tizayuca, Hidalgo. A total of 80 Holstein-Friesian calves under two months of age with symptoms of BRE (cough, runny nose, prostration, dyspnoea and weakness) were sampled. Two nasal swab samples were taken from each animal, one of which was placed in Amies transport medium, while the other was transported in a tube containing sterile PBS. The samples in Amies medium were processed for attempted isolation and culture of mycoplasmas in Friis liquid medium. From the nasal swabs transported in sterile PBS, DNA was extracted by heating the supernatants at 96°C. Molecular identification of the genus *Mycoplasma* was performed by nested PCR, which amplifies a 350-500 bp product corresponding to the 16S-23S intergenic spacer region of the ribosomal RNA. On the other hand, *M. bovis* species identification was performed by amplification of a 1626 bp sequence of the uvrC repair gene. *M. dispar* was identified by amplification of a 548 bp region of the rpoB gene, coding for the β-subunit of RNA polymerase. For the detection of *M. bovirhinis*, primers targeting a region of the rpoB gene, coding for the β-subunit of RNA polymerase, were used, obtaining an amplification product of 397 bp. Biochemical characterisation of the mycoplasma isolates and serological identification by metabolic inhibition test were also performed using reference antisera specific to different *Mycoplasma* species of bovine origin.

Results

Of the total samples processed for mycoplasma isolation, 41.2% (33/80) were positive, while 58.8% (47/80) were positive to the nested PCR used for *Mycoplasma* genus detection. By herd, 64.4% (29/45) of the samples from EdoMex were positive for Mycoplasma PCR, while 51.42 (18/35) of the herd from Tizayuca, Hidalgo were positive

for Mycoplasma PCR. The main species of *M. bovis* isolated and detected in the EdoMex herd was higher than in the Tizayuca herd, confirmed by serological identification and PCR. Meanwhile, *M. dispar* was the major species in the samples analysed from the latter herd. In both herds, infections with two or three species were identified in some of the samples tested. *M. bovis* and *M. dispar* were identified as the main species involved in respiratory disease in the study calves.

Conclusions

The use of end-point PCR allowed the detection of a higher number of mycoplasma infected animals from nasal exudates of calves with respiratory disease symptoms, indicating a higher sensitivity of the molecular tests used, relative to culture. The most commonly used method for the diagnosis of mycoplasmas is bacteriological culture; however, these bacteria are slow growing and have demanding nutritional requirements. In order to detect these pathogens more quickly, the use of polymerase chain reaction (PCR) has been proposed, as this technique facilitates the identification of infected animals, allowing field clinicians to take the necessary measures to control the spread of these microorganisms in the herds. In this study, the technique of PCR allowed the detection of a higher number of Mycoplasma-infected animals from nasal exudates of calves with symptoms of respiratory disease, indicating a higher sensitivity of the molecular tests used compared to culture.

1726 - FREQUENCY OF ASSOCIATION BETWEEN Mycoplasma bovis AND BACTERIA MEMBERS OF THE Pasteurellaceae FAMILY IN PNEUMONIA OF DAIRY CALVES.

Author: LAURA JARAMILLO MEZA, FERNANDO DÍAZ OTERO, ANABELLE MANZO SANDOVAL , LAURA HERNÁNDEZ ANDRADE

Objectives

Cattle production in Mexico is an important source of animal protein, since cow's milk constitutes 98% of the country's consumption. Additionally, milk and meat of this species represents 67% of the total agricultural products for domestic consumption. However, cattle production is efficiently affected by several factors, such as infectious diseases, and among them, respiratory problems represent worldwide economic losses. Calves pneumonia is a high-morbidity illness of housed dairy-type calves. It's considered is a multifactorial disease, that results from the interaction of infectious agents, environmental and management factors. Major bacterial pathogens involved include *Mannheimia haemolytica, Pasteurella multocida*, *Histophilus somni*, *and Mycoplasma bovis*. These bacteria are opportunistic pathogens as they can be isolated from the upper and lower airways of healthy cattle. However, *M. haemolytica* is widely regarded as the primary bacterial pathogen driving acute Bovine Respiratory Disease (BRD). While M. bovis is most commonly implicated in chronic cases of BRD, this agent's potential role in acute stages of BRD is unclear. The purpose of this study was to determine the frequency of interaction between Mycoplasma bovis and bacteria of the Pasteurellaceae Family in pneumonic processes in calves.

Material and methods

For this purpose, a bacteriological analysis of 47 samples of pneumonic lesions of calves collected at the slaughterhouse was conducted. For the isolation of mycoplasma, the samples were triturated with scissors to later inoculate test tubes containing Friis culture medium, making decimal dilutions, the tubes were incubated under microaerophilic conditions at 37 °C for 7 days, and then aliquots of each of the dilutions were inoculated in Friis solid medium to observe the development of the typical colonies. *M. bovis* isolates were identified by monospecific antisera using the metabolic inhibition test. At the same time, traditional bacteriological culture on 5% sheep blood agar was performed to isolate of *M. haemolytica* and *P. multocida*. Final identification of bacteria to species level was aided using the biochemical tests. DNA was extracted from the Mycoplasma-suspect cultures using a commercial kit. Molecular identification of the genus Mycoplasma was done by applying nested PCR, according to the method proposed by Harasawa in 1995, in which a 350 bp product corresponding to the 16S-23S intergenic spacer region of the ribosomal RNA is amplified. On the other hand, the identification of M. bovis species was performed following the protocol described by Subramaniam in 1998 in which a sequence of 1626 bp of the uvrC repair gene was amplified

Results

M. bovis was recovered from 38.3 % of the samples, in these cases 87 % was found associated with *Pasteurellacea*e, 39 corresponding with *M. haemolytica*, 43 % with *P. multocida* and 18 % with other bacterial genera. The high percentage of association of *M. bovis* with bacteria members of the *Pasteurellaceae* Family.

Conclusions

This urgently reveals the need to develop vaccines or immunogens against *M. bovis*, since traditional vaccines against bovine respiratory diseases do not contain them, this would greatly reduce the economic losses generated by the high rates of morbidity and mortality recorded by this type of disease in herds, together with the establishment of biocontainment and biosecurity measures. The successful development of protective M. bovis vaccines is still a long way off and much research is still needed in this area. Data on the present commercial vaccines in use today are modest at best, with one showing an efficacy of 1%. Clearly, improvements need to be made before control of this fast-emerging disease is possible. What is clear, however, is that any M. bovis vaccine needs to be part of a wider vaccination program involving other respiratory pathogens, including BVD, PI3V, Mannheimia, Pasteurella and possibly others. Hopefully, the use of bioinformatics tools will allow the proteomics analysis of the M. bovis secretome and consequently the detection of novel secreted proteins that can be used not only as diagnostic biomarkers, but also in the development of a potent vaccine for effective control of M. bovis infections.

1728 - Application of different vaccination protocols for the control of IBR in water buffaloes (Bubalus bubalis)

Author: Giovanna Cappelli , Carlo Grassi, Roberta Vecchio, Francesco Grandoni, Giovanna De Matteis, Giorgio Galiero, Esterina De Carlo, Stefano Petrini, Alessandra Martucciello, Alfonso Gallo

Objectives

Sia i bufali d'acqua che i bovini sono suscettibili all'infezione da alphaherpesvirus bovino 1 (BoAHV-1) e alphaherpesvirus bubalino 1 (BuAHV-1). Nel corso del tempo, questi due virus hanno subito un processo di “adattamento” per garantire un tasso di sopravvivenza più elevato nell'ambiente. Tuttavia, la latenza virale che caratterizza gli herpesvirus fa sì che l'animale infetto, anche se clinicamente sano, rappresenta un potenziale diffusore dell'infezione. Per questi motivi, la vaccinazione con marcatore è un mezzo efficace per controllare la rinotracheite infettiva bovina (IBR). Il presente studio si propone di fornire indicazioni sui diversi protocolli vaccinali per la specie bufalina contro l'IBR negli allevamenti di bufali d'acqua.

Material and methods

Lo studio si riferisce al periodo 2017-2021, in cui lo scopo era quello di utilizzare diversi protocolli di vaccinazione con marcatori per il bufalo indiano contro l'IBR. In particolare, abbiamo applicato la vaccinazione sistematica con marcatore IBR per valutare la sicurezza e l'efficacia dei due prodotti. In particolare, abbiamo utilizzato i seguenti vaccini: 1) vaccino con marcatore IBR inattivato (gE-); 2) vaccino con marcatore IBR vivo attenuato (gE-/tk-). I prodotti sono stati somministrati a bufali sieronegativi a BoAHV-1 e BuAHV-1.

Il primo protocollo vaccinale utilizzava due gruppi (A e B) di animali omogenei. Il gruppo A ha ricevuto un vaccino con marcatore IBR inattivato (gE-) a 0, 30, 210 e 240 giorni post-vaccinazione (PVD) per via intramuscolare (im). Il gruppo B rappresenta il controllo negativo. Al contrario, il secondo protocollo di vaccinazione ha utilizzato tre gruppi (C, D, E) di animali omogenei. In particolare, il Gruppo C ha ricevuto un vaccino con marcatore IBR vivo attenuato (gE-/tk-); Il gruppo D ha ricevuto un vaccino con marcatore IBR inattivato (gE-); Il gruppo E rappresenta il controllo negativo. I gruppi C e D a 0, 14, 20, 34 e 55 PVD sono stati iniettati per via im. Tutti i gruppi erano costituiti da cinque bufali d'acqua.

Trentacinque giorni dopo la prima vaccinazione, i gruppi A, C e D sono stati infettati con un ceppo BuAHV-1 di tipo selvaggio. Ciascun bufalo indiano ha ricevuto 5 ml X $10^{5,50}$ $TCID_{50}$ /ml per via intranasale. Successivamente, tutti gli animali sono stati valutati per i segni clinici e sono state ricodificate le temperature rettali. Inoltre, da ciascun animale sono stati raccolti tamponi nasali e campioni di siero da tutti gli animali, a 0, 2, 4, 7, 10, 15, 30 e 63 giorni dopo il test (PCD). Campioni di tamponi nasali sono stati utilizzati per due RTPCR contro BoHV-1 e BuAHV-1. I campioni di siero sono stati testati per anticorpi specifici mediante test competitivi gB-ELISA, gE-ELISA e di neutralizzazione del virus contro BoAHV-1 e BuAHV-1.

Results

Tutti i vaccini marcatori testati non inducono segni clinici o reazioni avverse. Le temperature rettali degli animali immunizzati sono rimasti entro i valori fisiologici. Un

aumento del titolo di BuAHV-1 è stato rilevato durante il periodo di vaccinazione nei gruppi A, C e D. Al contrario, la sieroconversione non è stata rilevata nei controlli non vaccinati. A 30 PVD è stato rilevato un sieropositivo GB nei gruppi vaccinati fino alla fine dello studio. Dopo l'infezione da provocazione, sono stati osservati segni clinici nei gruppi di controllo (B ed E). In particolare sono stati osservati ipersecrezione nasale, lesioni della mucosa nasale (pustole erpetiche) associate ad essudato mucopurulento e un leggero aumento della temperatura rettale (fino a 39,1°C). Si è verificata sieroconversione con aumento dei titoli anticorpali al giorno 14 PVD in tutti i gruppi vaccinati. Inoltre, negli animali vaccinati e di controllo è stato rilevato un sieropositivo gB durante tutto il periodo dell'infezione. Dopo l'infezione da test, è stata rilevata la positività alla PCR da 2-7 PCD. I bufali immunizzati hanno mostrato risultati gE-ELISA negativi durante il periodo di vaccinazione. Questo risultato mostra che né BuAHV-1 né BoAHV-1 circolavano tra gli animali durante la vaccinazione.

Conclusions

In conclusione, in questo studio, abbiamo dimostrato che la vaccinazione con marcatore IBR nel bufalo indiano era innocua ed efficace. Tuttavia, sono necessari ulteriori studi per studiare la capacità protettiva dei vaccini marcatori IBR vivi contro la latenza indotta dal virus di campo.

1733 - DISTRIBUTION AND VIABILITY OF Mycobacterium avium subspecies paratuberculosis IN SHEEP PRODUCTION UNITS.

Author: MARCO ANTONIO SANTILLAN FLORES, DIONICIO CORDOVA-LOPEZ, MARISELA LEAL-HERNANDEZ, EUGENIO VILLAGOMEZ-AMEZCUAMANJARREZ, JOSE VICENTE VELAZQUEZ-MORALES

Objectives
The objective of the work was to determine the distribution and viability of *Mycobacterium avium paratuberculosis* (Map) in different areas of the sheep pens.
Material and methods
Four samplings were carried out, every three months over a year (a sample for each climatic season of the year), in three UPPs of sheep with a history of paratuberculosis (prevalence >10%) and one UPP negative for paratuberculosis. From the pens, samples of feces present on the floor, sunny areas, pipes and puddles were taken, as well as from the feeders and waterers (indoor and outdoor). All fecal samples were analyzed by PCR-IS900 and bacteriological culture. With the results obtained, a frequency analysis was carried out.
Results
The samples taken around the feeders and waterers were positive for Map both in culture and PCR-a IS900 in the four samplings, but there was a higher frequency (50%-100%) in the samplings corresponding to summer and autumn. The viability of Map was detected from the samples taken from the sediment inside the waterers in 25-83%, in the sampling carried out in summer and autumn.
Conclusions
Identify areas and surfaces within the pens of sheep flocks infected with paratuberculosis, where there is a high accumulation and contamination with feces that allows Map to remain viable, is an important point to establish management measures to control the disease in herds.
Work funded by: INIFAP fiscal resources: SIGI-16593034896-P

1734 - OVINE PARATUBERCULOSIS: PREVALENCE AND RISKS ASSOCIATED WITH ANIMAL BREEDING STOCK AND MANAGEMENT OF FECES IN LIVESTOCK PRODUCTION UNITS.

Author: MARCO ANTONIO SANTILLAN FLORES, DIONICIO CORDOVA-LOPEZ, CLAUDIA CELIC GUZMAN-RUIZ, MARISELA LEAL-HERNANDEZ, EUGENIO VILLAGOMEZ-AMEZCUAMANJARREZ, JOSE VICENTE VELAZQUEZ-MORALES

Objectives
The objective of the study was to determine the prevalence and risk factors associated with paratuberculosis in relation to breeding stock and management of feces in the pens.
Material and methods
A cross-sectional epidemiological study was carried out. Sixty six Livestock Production Units (UPP) of sheep were worked in the state of Guanajuato México. A blood sample was taken per animal (n=1387) to carry out the Map serological diagnosis by agar gel Immunodiffusion, and UPP questionnaire was applied. All results were integrated into a database for epidemiological analysis using the STATA® 7 softwere.
Results
The UPPs that buy stallions had a prevalence of 42.4% and of females of 40% with an OR of 4 and 3. The UPPs that lend to stallions have a prevalence of 42.3% and an OR of 3. The UPPs that clean the pens in a period of time ranging from two months to a year, presented 45% and OR 4.3. In the UPP where feces remain accumulated, they had a prevalence of 66.6% and an OR of 30.
Conclusions
Health management should be focused on trying to reduce the presence of chronic infectious diseases such as paratuberculosis, since its slow spread and its chronic course in herds, puts the breeding stock and its productive and reproductive capacity at risk.
Work funded by: INIFAP fiscal resources: SIGI-16593034896-P

1743 - PCR detection of respiratory complex virus and bovine leukemia in vaccinated dairy herds.

Author: Ana Silvia González Méndez, Fernando Cerón Téllez, Jorge Luis Tortóra Pérez, Hugo Ramírez Álvarez

Objectives

Various viral agents that participate in the bovine respiratory disease complex (BRDC) have been described, together with *M. haemolytica* and *P. multocida*, however, little is known about the role that bovine leukemia virus (BLV) plays in coinfections with BRDC viruses. Viral infections can generate a significant negative impact on the reproductive efficiency of dairy herds. The consequences can vary from outbreaks of abortions, alteration of gestation, and early embryonic death, going unnoticed and undiagnosed. Bovine herpes virus 1 (BoHV-1) belongs to the *Herpesviridae* family and bovine viral diarrhea virus (BVDV) belongs to the *Flaviridae*family; they are part of the bovine respiratory viral complex (BRVC). BRDC is one of the main causes of morbidity and mortality in calves, together with the cost of treatment, it generates one of the main economic losses for the livestock industry. On the other hand, the bovine leukemia virus (BLV) belongs to the *Retroviridae* family, it is the causal agent of enzootic bovine leukosis, a contagious oncogenic lymphoproliferative disease of cattle, which is widely distributed in dairy herds in our country. The objective of the present study focused on the PCR detection of BoHV-1, BVDV, and BLV in dairy herds.

Material and methods

A group of samples from eight States of the Mexican Republic (Aguascalientes, Coahuila, México, Guanajuato, Jalisco, Puebla, Querétaro, and Tlaxcala) was analyzed with cooperating producers who applied vaccination against BRVC. Blood samples were obtained by puncture of the coccygeal vein in tubes with anticoagulant and subsequently centrifuged for phase separation [plasma and peripheral blood leukocytes (PBLs)]. The extraction of the genetic material from the PBLs (DNA and RNA) was carried out using a commercial kit. The identification of the viral agents of BVDV and BoHV-1 was carried out by the one-step multiplex PCR technique, using primers for each viral agent [1,2]. To determine BLV infection, PCR was used with specific primers that bind to a partial region of the envelope gene (*env*) previously described [3].

Results

A low prevalence of 3.81% was identified for BoHV-1, in contrast, the prevalence of BLV was 44.28%, and no BVDV-positive samples were identified.

Conclusions

There is little information on the molecular prevalence of BoHV-1, BVDV, and BLV; most studies in Mexico focus on determining serological prevalence and has been identified 26% of BLV in Mexican herds [4]; while in BoHV-1 42.8% seropositivity has been reported in the northwest of the country [5] and in the south of the country a 54.4% seroprevalence was estimated [6]. It is important to immunize animals to impact the prevalence of infections and to carry out constant molecular monitoring to determine the molecular prevalence of BoHV-1 and BVDV since many studies carried out in the country have focused on the detection of antibodies, which does not allow differentiating vaccinated animals from infected ones.

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1744 - Development of an ELISAi based on recombinant proteins for the diagnosis of BLV

Author: Hiram Sánchez Gasca , Ana Silvia González Méndez , Julieta Sandra Cuevas Romero, José Luis Cerriteño Sánchez, Hugo Ramírez Álvarez

Objectives

Bovine leukemia virus (BLV) is the causal etiological agent of enzootic bovine leukosis (EBL), a silent and neglected disease that lasts a lifetime and mainly affects dairy cattle, and is widely distributed worldwide. Standardization of an indirect ELISA based on recombinant protein (p24-p12) capsid-nucleocapsid for the detection of antibodies against bovine leukemia virus (BLV).

Material and methods

Seven hundred blood samples were obtained from Holstein cattle from nine states of Mexico. To know your serological status, a commercial ELISA kit was used that detects antibodies against the BLV gp51 protein. For the expression of the recombinant protein, the complete coding sequence of BLV capsid and nucleocapsid (p24-p12) of the gag gene of genotype 1 was amplified by PCR from an infected cow. An ELISA test based on using the recombinant BLV protein (p24-12 BLV) was standardized in 96-well plates. Those discordant samples between the ELISAs were tested by the SDS-PAGE and western blot technique; the transfer was done on PVDF (polyvidone fluoride) membranes.

Results

An 851 bp fragment was expressed corresponding to the gag gene of BLV genotype 1 that encoded the capsid and nucleocapsid proteins. The recombinant p24/p12 protein consistently migrated as a 71 KDa molecular weight band, which was confirmed by bioinformatics programs. The immunogenicity of the p24/p12 protein was analyzed in ELISA tests (ELISA-p24/p12) using 700 bovine samples previously evaluated with a commercial ELISA. In this first stage, the sensitivity and specificity of the ELISA-p24/p12 were 82.8% and 63.8% respectively, taking the commercial ELISA as the gold standard. Of the discordant samples (n=152) their immunoreactivity was demonstrated by WB, determining a sensitivity of 86.7% and a specificity of 82.4%, presenting an adequate degree of agreement between ELISAs.

Conclusions

BLV infection in cattle around the world varies widely, in Mexico prevalence studies have not been carried out adequately since not all regions of the country have been sampled and the available studies are of opportunity, and in some cases the samples are limited (4). In the present study, a combined recombinant protein was expressed involving the capsid and nucleocapsid of BLV. The serological tests have been used extensively to identify cattle infected with BLV due to their rapidity, cost-effectiveness, and ease of interpretation(1). The commercial serological tests for the detection of BLV infection are based on the identification of antibodies against the glycoprotein gp51 of the viral envelope and/or against the capsid protein p24, since these proteins are the main ones that are recognized by the immune response (3,5). In some studies, in naturally infected animals antibodies against p24 are found less frequently or in lower titers than antibodies against gp51, this may be related to antibodies against gp51 appearing early and at higher titers compared to antibodies formed against the p24 protein (2). On the other hand, two immunodominant epitopes have been identified in the BLV p24 protein,

in contrast to five epitopes in the gp51 protein (5). Nevertheless, the ELISA-p24/p12 test can identify the presence of antibodies against BLV in animals that were negative in the ELISA commercial (2,5). The glycosylated recombinant proteins, such as gp51, require the use of expression systems in eukaryotic cells, which leads to the use of more complex techniques; however, recombinant proteins p24/p12 do not require post-translational processes and could be generated in bacteria, which impacts a lower cost and in addition to the use of antigens from a BLV genotype prevalent in infected animals in the country are highly preferable.

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1745 - Identification of genotype 1 of the bovine leukemia virus (BLV) in dairy herds in the country.

Author: Ana Silvia González Méndez , Fernando Cerón Téllez , Rosa Elena Sarmiento Silva, Jorge Luis Tórtora Pérez , Hugo Ramírez Álvarez

Objectives
Bovine leukemia virus (BLV) belongs to the order Ortevirales, family Retroviridae, subfamily Orthoretrovirinae, and genus Deltaretrovirus. It is an oncogenic retrovirus and the causative agent of enzootic bovine leukosis, a contagious lymphoproliferative disease of cattle. There are twelve genotypes so far identified with worldwide distribution; in Mexico, there is little information on the matter. The objective of the study was to identify the prevalence of the bovine leukemia virus and the genotypes present in dairy herds in the country.
Material and methods
275 whole blood samples with anticoagulant were collected from cattle in the States of Aguascalientes, Coahuila, Mexico, Guanajuato, Jalisco, Puebla, Querétaro, and Tlaxcala. The samples were centrifuged to separate plasma and peripheral blood leukocytes (PBL). The plasma was evaluated by a commercial ELISA to determine the presence of antibodies against BLV and the genetic material (DNA) was extracted from the PBL; which was used to determine the BLV proviral DNA by PCR with specific primers of the env gene, the amplicons were purified with a commercial kit and sent for sequencing.
Results
A seroprevalence against BLV of 66.18% and a prevalence with PCR of 56.73% was found, with the State of Querétaro being the region with the highest prevalence 21.09% by ELISA and 15.64 by PCR, followed by Tlaxcala (13.09 and 90.9.%), Coahuila (12.36% and 12.36%) and Aguascalientes (11.27 and 10.55%) by ELISA and PCR respectively. 18 sequences were obtained, which were analyzed by Blastn to confirm similarity. The sequences were used to build a phylogenetic tree which included reference sequences to determine the BLV genotype of the sequenced samples, finding that the 18 sequences (10 from Queretaro, 3 from Coahuila, 3 from Guanajuato, 1 from Tlaxcala and 1 from Aguascalientes) were associated with BLV genotype 1.
Conclusions
BLV affects cattle and is present in cattle worldwide with relatively high genetic diversity. In the study by Cordero-Pulido et al., (2023) they identified a prevalence of PCR in dairy and dual-purpose cattle in the State of Veracruz 6.96%, in contrast, In the study by Cerón et al. (2020) (Cerón et al., 2020), 405 samples were evaluated, determining a serological prevalence of 44.1%, data lower than those obtained in our study, which is possible to be related to the number of samples used in the present study; However, the high prevalence identified is highly striking, which may be a direct consequence of the lack of interest or ignorance of producers regarding the prevention measures that they can implement to control the infection. In the works of Heinecke et al., (2017) and Cerón et al., (2020) also found BLV genotype 1 to be prevalent; however, it has not been described whether BLV genotypes have pathogenic differences, although the clinical presentations of lymphocytosis and development of Lymphomas are frequently identified in infected cattle (Montero et al., 2022). It was identified that BLV genotype 1 was the only one present in the samples studied. A high prevalence of infection was

determined in different livestock regions of the country. Despite the studies that have been done on BLV infection and to reinforce the findings, a larger number of samples from other regions of the country and from other production systems, for example, cattle for meat production, are necessary to determine the real prevalence of BLV in Mexico.

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1760 - Immune protection induced by E2 recombinant glycoprotein of bovine viral diarrhea virus in a murine model

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Objectives

Bovine viral diarrhea virus (BVDV) is considered the most important viral pathogen in ruminants worldwide due to the extensive range of clinical manifestations exhibited by infected animals. Vaccination can prevent reproductive failure and respiratory and gastrointestinal disorders caused by BVDV infection, leading to significant economic losses in the beef and dairy industries of various countries. However, considering their limitations, conventional vaccines such as live, attenuated, and killed viruses have been applied. Hence, different studies have described subunit vaccines as an effective and safe alternative for BVDV protection. Therefore, in this study, the ectodomain of E2 (E2e) glycoprotein from NADL BVDV strain was expressed in mammalian cells and used in two vaccine formulations to evaluate immunogenicity and protection against BVDV conferred in a murine model.

Material and methods

The study was performed in specific-pathogen-free Balb/c female mice 6- to 8-week-old (n=6). All animals were maintained under pathogen-free conditions and handled in strict accordance with the guidelines and protocols approved by the Care and Use for Experimental Animals Sub-committee at the Facultad de Medicina Veterinaria y Zootecnia-Universidad Nacional Autónoma de México (FMVZ-UNAM). Mice were randomly allocated to each of the five treatment groups and intraperitoneally immunized as follows: Group 1 was immunized three times with 50mg of BVDV recombinant protein E2e; Group 2 was immunized three times with 50mg of BVDV recombinant protein E2e formulated in MontanideÔ ISA 61 VG; Group 4 was inoculated three times with physiological saline solution (PSS) emulsified with MontanideÔ ISA 61 VG; Group 5 was inoculated with PSS and served as a negative control group. Immunization was performed on days 1, 15, and 30. Additionally, group 3 was included as a positive control for the BVDV challenge. Subsequently, to evaluate the conferred protection against BVDV, mice were challenged six weeks after the third immunization; therefore, RT-PCR, histopathological assessment, and immunochemistry in tissue samples of the lung, stomach, liver, spleen, intestine, kidney, and brain were conducted. In addition, the humoral immune response was evaluated after vaccination and challenge. The significance of the differences in neutralizing antibodies titer was estimated by the Student's T test performed in Graph Pad software 6.0 version (Graph Pad Prism, Software Inc. La Jolla CA). The significant difference in tissue immunopositivity among the immunized and control groups was determined by one-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test performed in JMP software 11 version.

Results

After the first treatment inoculation, vaccinated mice from groups 1 and 2 seroconverted, developing a stronger neutralizing humoral response than the positive control group 4 (p <0.001). Notably, the vaccination using E2e + ISA 61 VG elicited a

significantly higher neutralizing antibodies level in a short period than group 1 (p <0.001). No significant differences were found between groups 3 and 4. Data analysis revealed a significantly stronger neutralization activity in groups 1 and 2 compared with the BVDV NADL control group. In addition, immunization using E2e + ISA 61 VG prevents animals from developing severe lesions in surveyed tissues. Moreover, this group acquired protection against the BVDV challenge, evidenced by a significant reduction of positive staining for BVDV antigen in the lungs, liver, and brain between the experimental groups.

Conclusions

Our findings demonstrated that using E2e + ISA 61 VG and boost strategy induces greater BVDV protection by an early humoral response and reduced histopathological lesions and BVDV antigen detection in affected organs, indicating that E2e + ISA 61 VG subunit formulation provided protection against BVDV challenge in mice than solo E2e immunization; therefore, the E2e + ISA 61 formulation represents a viable vaccine candidate to be evaluated for the target species.

1772 - 3M Strategy for Controlling Emerging and Enzootic Diseases in Cattle Breeding, Tuberculosis Case

Author: said jaajaa jaajaa, Wafa Bouglita, Tarek Albouchi , Khaled El Hicheri, Ramzi Boubaker ELandolsi

Objectives

The prevalence of tuberculosis affects 30% of the Tunisian livestock, and this failure in the control of this zoonosis forces us to find new methods of control. The combination of surveillance with innovative “Monitoring” techniques and inspection of risk factors in “Managment” and monitoring through a “Mode-tech” by application and artificial intelligence could help to understand causality and predict future, which gives more agility in this approach, also called the “3M strategy”.

Material and methods

To do it, a survey was carried out on a farm of 72 dairy cows with a positivity rate by intradermal reaction IDR of around 2% plus a doubtful 22% with a concordance of two tests 2 months apart. A trial using three types of Lateral Flow Device (LFD) tests, two for Ac and one for Ag, from two different brands, “one Indian and two Chinese”, was conducted and aimed to provide more information on the added value of these techniques based on the detection of the MPB70 antigen specific to the tuberculosis complex, especially *M.bovis*. The results of the different tests were compared against a reference test, the PCR targeting the MPB70 gene of *M. bovis* and IS900 P 90/91 of *M. avium paratuberculosis*, in order to evaluate the precision of conventional techniques compared to rapid tests, but also these last among themselves.

Results

The positivity by “LFD” tests is around 27%, with good concordance between Ac tests. Also, doubtful cases by intradermal reaction “IDR” were revealed as positive cases by rapid tests (81%). In addition, the results of Ag tests show a positivity rate of around 20%.

The report from the farm audit proves the introduction of the germ due to the exchange of equipment with a contaminated farm. A weakening of the immune system of cows has been noted by heat stress factors due to conception as well as self-medication with the same instrument of injection.

The development of a “SafaFarme” application makes it possible to have a data base about tuberculosis disease cases and quantification of failed risk factors in management, and to provide a report objective on the same day, and to establish a roadmap of recommendation and remote monitoring of the mycobacterium infection in the animal and the environment in order to act in appropriate and rapid ways.

PCR results are pending; normally, the result will be in two weeks, and the final report is after studying the big data by artificial intelligence (AI)

Conclusions

The valorization of innovative technologies in diagnostics like LFD and PCR and the resolution of big data about animals and the environment by application can be useful to control emerging and enzootique disease like tuberculosis, but there are other prophylactic measures like a regional scale, such as a medical work unit, and medical prophylaxis such as vaccination, such as auto-vaccination that can be important in this situation.

1775 - Reduction of hematophagous bat aggressions with the use of systemic vampiricide in a population of the state of Veracruz, Mexico

Author: Selene Piscidia García Sarabia, María Luisa Mendez Ojeda, Vicente Eliezer Vega Murillo, Raymundo Salvador Gudiño Escandón

Objectives

Rabies is an infectious disease caused by a virus belonging to the family Rhabdoviridae, genus Lyssavirus, it is a zoonosis that affects all domestic and wild mammals or even humans, transmitted through contact with infected saliva by bites or scratches. In the case of bovines, bovine paralytic rabies (RPB) is generally transmitted mainly by the hematophagous bat (Desmodus rotundus) (MH), which, if infected, transmits the rabies virus by feeding on its blood. SENASICA has a National Campaign for the prevention and control of rabies in cattle and livestock species (NOM-067-ZOO-2007), which deals with notifications of suspect animals and confirms them by means of diagnosis in official and authorized laboratories; this campaign is currently in the control phase, It covers a total of 26 states in Mexico, which are characterized by the existence of the hematophagous bat Desmodus rotundus, the main reservoir and source of infection for livestock, as well as the environmental conditions conducive to its development. The state of Veracruz meets these conditions in addition to a large livestock inventory, with conditions conducive to the presentation of RPB, it is worth mentioning that from 2020 to 2023 it was the state that presented more rabies outbreaks in Mexico. The objective of this study was to evaluate the reduction of bites caused by hematophagous bats in cattle in the locality of La Tasajera in the municipality of Medellin, Veracruz, Mexico.

Material and methods

A systemic vampiricide approved by NOM-067 and based on Warfarin at a rate of 5mg/kg via IM as a control method was used. The information was analyzed with a paired samples t-test to determine the effect before and after the application of the systemic vampiricide. Twenty-one producers in the area collaborated in an organized manner, first a talk was given on the subject and the work was coordinated, so that on a synchronized day before treatment with the systemic vampiricide.

Results

A total of 683 cattle were checked, two data were considered, number of animals bitten and number of bites per animal, of which 255 presented bites caused by MH, which represents 37.3%.For the number of bites, in these 255 cattle, a total of 777 bites were counted, which represents 1.1 % bites per bovine in the herd. The systemic Vampiricide was applied only to the cattle that presented bites, a second revision was made to all the cattle 10 days after the treatment, time that Warfarin is present in the bloodstream and the product acts, in this second revision 62 animals were found bitten and a total of 108 bites, which represents a 75.7 % reduction ($p=0.02$) of animals bitten and 86.1 % ($P < 0.05$) of reduction in the number of bites.

Conclusions

The use of systemic vampiricide is a good tool to reduce aggressions by hematophagous bats and at the same time to reduce the risk of RPB. It is important to involve producers in the work and training to prevent the disease and to control the MH population without harming other types of beneficial bats.

1783 - Pathogenomics of the respiratory Mycoplasma bovis strains circulating in cattle

Author: Ethan Dudley , Sal Lamsal , Paul Morley, Matthew Scott, Lee Pinnell, Hatem Kittana, Alexis Thompson, Robert Valeris-Chacin

Objectives

Bovine respiratory disease (BRD) is a major economic and animal welfare issue in the beef industry, leading to a nearly one billion dollar loss in the United States annually . It is a multifactorial disease caused by the interaction of several factors such as stress from weaning and transportation, commingling, and infection by several viruses and bacteria. *Mycoplasma bovis* is one of the main bacterial pathogens associated with BRD, especially with chronic cases. However, there is a lack of information about the genomic characteristics of the strains circulating in the Texas panhandle due to the difficulties of isolating *M. bovis* in clinical specimens. Therefore, the objective of this study is to describe the genomic features of field *M. bovis* strains isolated from cattle in the Texas panhandle.

Material and methods

Fifty-four isolates displaying mycoplasma-like growth were recovered from bovine lung lesions by the Texas Veterinary Medical Diagnostic Laboratory in 2021 and 2022. Of these isolates, 33 were determined to be *Mycoplasma bovis* via species-specific qPCR (using the *uvrC* gene). The DNA of these isolates was extracted using the DNeasy Ultraclean microbial kit (Qiagen). Whole genome amplification was used to improve DNA yields utilizing the REPLI-g Midi kit (Qiagen). Whole genome sequencing was carried out using the native barcoding kit v14, R10.4.1 flow cells, and the MinION Mk1C sequencer, all from Oxford Nanopore Technologies. Sequence quality was assessed using LongQC, and *de novo* assemblies were derived using Flye. Assemblies were annotated using Prokka. The presence of single nucleotide polymorphisms (SNPs) in the core genome was inferred using Snippy. Roary was used to estimate the pan-genome. The pan-genome was then associated with host characteristics using Scoary. Abricate was used to search the isolates for virulence and antimicrobial resistance genes.

Results

Our results showed significant clustering of isolates based on operation of origin and age of the animal. We found the ISMbov1 insertion sequence, which has a close sequence similarity with an insertion sequence in *M. agalactiae,* in several of our isolates. Our isolates share three common virulence factors. Elongation factor Tu and phdB alpha-ketoacid dehydrogenase subunit beta are both responsible for the ability of *Mycoplasma bovis* to attach to the respiratory epithelium in cattle. P48 BMP family ABC transporter substrate-binding protein is a known monocyte activation/differentiation factor, helping to initiate the immune response to *Mycoplasma bovis*. Only one of our isolates possessed an antimicrobial resistance gene, the TET44 tetracycline resistance ribosomal protection protein.

Conclusions

Our results indicate that there is a great deal of genetic diversity among our *Mycoplasma bovis* isolates, despite their shared geography in the Texas Panhandle. We detected the three commonly shared virulence factors among all of our isolates, all implicated in host

epithelial attachment and immune activation. The prevalence of antimicrobial resistance genes was low, with only one isolate testing positive for a gene conferring tetracycline resistance.

1786 - ISOLATION, IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF MAJOR MASTITIS-CAUSING BACTERIA IN DAIRY FARMS, WOLAITA SODO TOWN, SOUTHERN ETHIOPIA

Author: Feven Nigatu Sharecho

Objectives

General objective

The objective of this study is to isolate the major mastitiscausing bacterial species and determine antibioticsusceptibility profiles of the isolates in selected dairy farms inSodo , Wolaita, Ethiopia.

Specific objectives

➢ To identify major bacterial species from mastitic cow milksample in the area.

➢ To assess the antibiotic susceptibility profile of the isolates.

Material and methods

Study Areas

This study was conducted in Wolaita Sodo Town, WolaitaZone. Wolaita Sodo Town is t he capital town of Wolaita Zonelocated in southwest of Addis Ababa and is located abo ut 329km away from to Addis Ababa, the capital city of Ethiopia. Itlocated between 6°4 ´N to 7°1´N latitudes and 37°4´E to 38°2´Elongitudes (CSA, 2020). The altitude of wolait a Zone variesfrom 11002950 m.a.s.l. with a mean annual temperature ofabout 20°C. T he rainfall pattern over much of the area istypically bimodal with the big rainy season e xtending fromJune to September and a small rainy season occurring fromFebruary to A pril. The mean annual rainfall of the area rangesfrom 4501446 mm with the lowest bei ng in low land and thehighest in high land. The livestock population in the area isestim ated to be 68,900 cattle, 1992 sheep, 382 goats, 121horses, 131 mules, 488 donkeys, a nd 55,191 chickens (WolaitaZone Agricultural Office, 2020).

Study Animals

The study animals were lactating Local Zebu, Jersey andHolstein Friesian (HF) cross bre eds kept under semi- intensivemanagement system in Wolaita Sodo town. In this study allage categories, parity, lactation stage, breed, and bodycondition of animals were re corded. Accordingly, the age ofanimals was determined from birth records and categor ized asyoung (3–6 years), adults (7
9 years), and old (≥ 10) (Pace andWake, 2003). Body condition score was categorized a s good, medium, and poor as described by Nicholson and Butterworth,(1986).
Parity was divided into three categories: as few (with ≤ 3calves), moderate (4-
7 calves), and many (> 7 calves). Thecow's lactation stage was divided into three categ ories: asearly (1–4 months), mid-stage lactation (4–8 months), andlate-
stage lactation (above 8 months to the beginning of thedry period). In addition, the far ms were categorized into small,medium, and large-
scale dairy farms based on the number ofdairy cows in the farm, large-
scale (>30 dairy cows), medium-scale (>5≤30 dairy cows), and small-
scale (≤5 dairy cows)dairy farms. They were milked by hand twice a day at the tiestall.

The parity and lactation stage of the cow was classified asdescribed by in the work of F esseha et al., (2021); Biniam etal., (2019); Tesfaheywet and Girma, (2017). Lactating co wsthat haven't received any antibiotic treatment (≤ 1 month)were included. On other

hand, cattle that have recentlyreceived antibiotic treatment were excluded from this st udy.All these data were recorded appropriately and kept in datarecord sheets for analy sis.

Study design

A cross-
sectional study design was conducted to isolate andidentify major bacterial species cau sing mastitis from rawmilk samples of lactating dairy cattle. The purpose of thisstudy w as explained to dairy owners and the fresh raw milksamples were collected from their dairy cow's based on theirwillingness.

Sample Size Determination

The data on the total dairy farms located in Wolaita Sodo townwere taken from Wolait a Sodo Regional Agriculture andlivestock resource development office. Accordingly, a t otal of116 dairy farms were registered by the office where 65 weremanaged under se mi-intensive and 51 were managed underextensive farming system.

For this study purposes, out of sixty-five semi-
intensivelymanaged dairy farms, a total of 40 dairy farms werepurposively selected bas ed on accessibility of transportationfor sample taking, willingness of the farm owners t oparticipate in the stud and those lactating cows which didn'treceive antibiotics treat ment within one month during thestudy time. Thus, a total of 150 lactating dairy cows wereavailable on the selected farms based on the inclusion criteria.

Study Method

Physical examination of the udder and milk

Physical examination for evidence of clinical mastitis wasconducted in all lactating cow s that were sampled during thestudy time. Udders of cows were examined visually and bypalpation for any presence of clinical mastitis. During theexamination, attention wa s paid to cardinal signs ofinflammation, blindness, injuries, milk clots, symmetry, size,c onsistency of udder quarters, swelling and abnormal milk,pain in the udder, swelling, r edness, change in milk quantityand quality and take all vital sign of each animal (Rados tits etal., 2007). A cow was considered to have clinical mastitis if itfulfilled at least two of the clinical findings, (1) pain reactionupon palpation, (2) changes in color and consist ency of milk(blood-
tinged milk, watery secretions, clots, pus) and (3)change in consistency of the udder (L akew et al., 2009).

California Mastitis Test

After physical examination, all selected cows were tested bythe California mastitis test (CMT). Subclinical mastitis wasdiagnosed based on CMT results and the nature of coag ulationand viscosity of the mixture, which shows the presence, andthe severity of the i nfection, respectively (Radostits et al.,2007).

The udder was washed thoroughly and dried, Teats weredisinfected with cotton soake d in a 70% alcohol solutionbefore sampling, after discarding the first 2-
3 milking streamsas used by (Moges et al., 2011). A squirt of milk, about 2 mlwas drop ped in the wall of each side of the paddle and thesame amount of CMT reagent was ad ded to it. moving gentlyand thoroughly to mix well then see the gel formation. CMTgra des were evaluated and the result was scored based on thegel formation and categoriz ed as negative if there was no gelformation, or positive if there was gel formation (Kerr o andTareke, 2003).

The time chosen for milk sampling was before milking. Teatsthat identified as positive f or clinical and subclinical mastitiswere considered for milk sample collection. Approxim ately5ml milk were collected aseptically with sterile universaltubes with tight-fitting cups used after discarding the firstthree milking streams (Moges et al., 2011). Th en sample fromeach quarter was collected separately and recordedaccordingly the nea r teats were sampled first and thenfollowed by the far ones and transported in the iceb ox with icepacks to the Microbiology Laboratory of the School ofVeterinary Medicine, Wolaita Sodo University. After arrival atthe laboratory, the samples were immediately cultured and orstored at+ 4°C for a maximum of 24 h until culturing onstandard bacteri ological media (Quinn et al., 2002). Theexamination of udder and milk and sample colle ction wasperformed in this way.

Isolation and Identification of bacterial species

The bacteriological analysis of milk samples from bothclinical and subclinical quarters was performed using thestandard bacteriological protocols (Quinn et al., 2002; Markey et al., 2013). Milk samples obtained from each teat quarterwere cultured using 7% defi brinated bovine blood on bloodagar bases followed by MacConkey agar then incubate daerobically at 370C for 24-48 hours. Then, plate growth,morphology, and hemolysis pattern on the blood agar ba sewere subsequently studied. Subcultures were made for thepure identification of isol ates.

The growth of bacteria on mannitol salt agar and purple agarwere used to identify Stap hylococci species. The fermentationof mannitol by S. aureus causes yellow discoloratio n of themedium. Colonies that show weak or delayed yellow color onMannitol Salt Aga r (MSA) after 24 hrs. of incubation wereconsidered as Staphylococcus species and colo nies that failedto produce any change on the medium were determined asCoagulase N egative Staphylococcus species (CNS) (Quinn etal., 2002).

The detection of Streptococci species was performed onEdwards's media according to their growth characteristics.Different biochemical tests such as Tube coagulase test,cat alase test, CAMP test, indole production, methyl red test,Voges-Proskauer reaction, urease production, citrateutilization, and sugar fermentation were used to identify theStaphylococci and Streptococci species.

Also, pink-colored presumptive E. coli colonies were sub-cultured onto Eosin Methylene Blue (EMB). Colonies with ametallic green sheen on EM B were later characterizedmicroscopically using Gram's stain. Presumed E. coli colonieswere then transferred onto nutrient agar for furtheridentification using bi ochemical tests. Oxidase reaction,Catalase testing, Triple Sugar Iron (TSI), "IMViC" (ind ole,methyl red, Voges-Proskauer, and citrate) test, and motilitytest have been used to identify the E. coli species (Quinn etal., 2002). Bacterial isolates were identified based on colonychara cteristics and the presence of hemolysis gram staining,catalase test, coagulase test, oxi dase test, and Indole, Methylred, Citrate tests, CAMP test (Cheesbrough, 2006).

Antimicrobial Susceptibility testing

The antibiotic susceptibility tests of the isolates wereperformed according to the Natio nal Committee for ClinicalLaboratory Standards (NCCLS) method using Kirby-Bauerdisk diffusion test on Muller-Hinton agar. The agar diskdiffusion method has been used to test common fast-

growingbacterial pathogens and recognized to work well with bacterialisolates. Reliable results can be obtained with disk diffusiontests that use standardized methodology and zone diametermeasurement correlated with minimum inhibitoryconcentration (MIC) and the behavior of strains amongclinically susceptible and resistant categorizations (CLSI,2020).

The isolates were tested against for five namely Penicillin (G)(30µg), Tetracycline (TE) (30µg) erythromycin (E) (15µg),Ciprofloxacin (CIP) (30µg) and Gentamycin (CN) (30µg)were tested. Pure colonies on nutrient agar were taken with awire loop and transferred to a tube containing 5 ml of 0.85%Saline water and emulsified. The broth culture was incubatedat 37°C for 4 hours until it achieved the 0.5 McFarlandturbidity standards. Sterile cotton swab was dipped into thesuspension and the bacteria were swabbed uniformly over thesurface of Muller- Hinton agar plate within a sterile safetycabinet. The plates were held at room temperature for 15minutes to allow drying. Antibiotic discs with knownconcentration of antimicrobials were placed and the plateswere incubated for 24 hours at 37°C.

Results

Prevalence of Clinical and Subclinical Mastitis

A total of 150 lactating cows were examined using clinicalexamination and CMT tests to detect clinical and sub-
clinicalmastitis in this study. The overall prevalence of mastitis was48.6% (73/150); of which 17.3% (26/150) were clinicallyinfected and 31.3% (47/150) had subclinical mastitis

Prevalence of mastitis in quarter level

A total of 600 quarters were examined from 150 lactatingcows and 1.8% (11/600) of the teats were blind. On the other hand, 13.8% (83/600) of the quarter were diagnosed with
mastitis. Accordingly, the prevalence of clinical andsubclinical mastitis at the quarter level was 4.7% (28/600) and9.6% (55/572), respectively (Table 1).

Table 1: Prevalence of mastitis in quarter

Prevalence of mastitis in quarter level

A total of 600 quarters were examined from 150 lactatingcows and 1.8% (11/600) of the teats were blind. On the other hand, 13.8% (83/600) of the quarter were diagnosed with mastitis. Accordingly, the prevalence of clinical and sub clinical mastitis at the quarter level was 4.7% (28/600) and9.6% (55/572), respectively (Table 1).

Table 1: Prevalence of mastitis in quarter level

Quarter	No. of Examined	No. Positive animals	Prevalence (%)
Right front	150	14	9.3.
Left front	150	18	12
Right rare	150	21	14
Left rear	150	30	20
Total	600	83	13.8

Isolation rate of major mastitis-causing bacteria

In this study, from the total of 72 raw milk samples examinedfor isolation of major mastitis-
causing bacteria species, 94.4%(68/72) of the milk samples were harboring different types ofbacterial pathogens. while. In the current study, a total ofbacterial isolates with

a frequency of 105 were identified.Thus, 71.4% (75/105) gram-positive and 28.6% (n=30) gram-negative bacteria were isolated. Over 68 bacteria of fivegenera were isolated. Then, of the total isolates, bothcontagious pathogens and environmental pathogens wereinvolved. Major bacteria isolated in this study wereStaphylococcus aureus, other coagulase-negativeStaphylococcus species (CoNSs), Streptococcus agalactia,other Streptococci species, and E.coli accounting for aproportion of 40% (42/105), 9.5% (10/105) 16.2% (17/105),5.7% (6/105) and 28.6% (30), respectively.

Table 2: The overall prevalence of mastitis with risk factors

Risk factors	Category	No of examined	No. of positive	Prevalence (%)
Age	Young	54	25	46.3
	Adult	50	18	36.0
	Old	46	30	65.2
Breed	Local	22	7	31.8
	HF Cross-breed	100	56	56.0
	Jersey	28	10	35.7
Lactation stage	Early	60	32	53.3
	Medium	52	25	48.2
	Late	38	16	42.1
Udder size	Small	44	17	38.6
	Medium	57	26	45.6
	Large	49	30	61.1
Teat & udder injury	Yes	43	28	65.1
	No	107	45	42.0
Parity	Few	59	23	38.9
	Medium	70	34	48.5
	Many	21	16	76.1
Farm size	Small	49	26	53.1
	Medium	50	22	44
	Large	51	25	49

Bacterial isolates frequency in sub-clinical and clinicalmastitis

The rate of isolation of S. aureus bacteria was highest at27.6% in subclinical mastitis and 12.3% in clinical mastitis,while E. coli had a high prevalence of clinical mastitis 16.1than that of sub-clinical mastitis 12.3% (Table 3).

Table 3: Isolates frequency in sub-clinical and clinical mastitis

Bacterial isolates	Clinical mastitis		Sub-clinical mastitis	
	Frequency	Prevalence (%)	Frequency	Prevalence (%)
S. aureus	13	12.3	29	27.6
CoNs	0	0	10	9.5
Strep. agalactia	11	10.4	6	5.7
Strep. Species	0	0	6	5.7
E. coli	17	16.1	13	12.3

CoNs=Coagulase-negative staphylococcus species; spp. =Species

Antibiotic susceptibility test in isolates of bacteria

Staphylococcus aureus isolates were susceptibility forGentamicin (90.4%) followed by erythromycin (85.7%) andCiprofloxacin (71.4%), whereas relatively lower susceptibility was seen for penicillin (23.8) and tetracycline (19%). On theother hand, CoNSs and Streptococcus species exhibitedsusceptibility to gentamicin (82.4% to 100%), Ciprofloxacin(71.4% to 100%) and erythromycin (66.6% to 85.7%). Inaddition, E. coli isolate were highly susceptible tociprofloxacin and gentamycin. On other hand, the isolateswere highly resistance to Penicillin and tetracycline (Table 4).

Table 4: Antibiotic susceptibility test in isolates of bacteria

Bacterialisolates	Susceptibility pattern (%)	Antibiotics disc tested				
		G (30 ug)	CN (30 ug)	CIP (30 ug)	E(15ug)	TE (30 ug)
S. aureus (n=42)	S (%)	10(23.8)	38(90.4)	30(71.4)	36(85.7)	8(19)
	I (%)	0(4.8)	4 (9.5)	02(0)	4(9.5)	0(0)
	R (%)	32(76.1)	0(0	12(28.6)	2(4.8)	34 (81)
CoNs (n=10)	S (%)	0(0)	10(100)	8(80)	7(70)	0 (0)
	I (%)	3(30)	0(0)	2(20)	2(20)	2(20)
	R (%)	7(70)	0(0)	0(0)	1(10)	8(80)
Streptococcusagalactia(n=17)	S (%)	0(0)	14(82.4)	17(100)	12(70.6)	3(17.6)
	I (%)	0(0)	3(17.6)	0(0)	0(0)	0(0)
	R (%)	17(100)	0(0)	0(0)	5(29.4)	14 (82.4)
Streptococcusspp (n=6)	S (%)	0(0)	5(83.3)	6(100)	4(66.6)	1(16.7)
	I (%)	0(0)	1(16.7)	0(0)	2(33.3)	0(0)
	R (%)	6(0)	0(0)	0(0)	0(0)	5(83.3)
E. coli (n=30)	S (%)	5(16.6)	28(93.3)	26(83.3)	17(57.7)	7(23.3)
	I (%)	0(0)	2(6.7)	0(0)	4(13.3)	2(6.7)
	R (%)	25(83.3)	0(0)	4(13.3)	9(30)	21(70)

S-Susceptible, I-Intermediate and R-Resistance; Gentamicin (CN), Erythromycin (E), Ciprofloxacin (CIP), Penicillin (G)and Tetracycline, (TE)

Conclusions

The current study revealed that the prevalence of mastitis atcow and quarter levels was found to be relatively high in thest udy area. There is a concern of antibiotic drug resistance formost of the tested drugs that were commonly used in the studyarea. Moreover, most of the dairy cows were infected with subclinical type of mastitis and the two genera and threespecies of bacterial species were isolated from both clinical and subclinical mastitis cases. Factors such as age, breed, farm size, lactational stage, parity, udder size and injury to udder ofdairy cows have contributed for occurrence of mastitis in the dairy cows. On the other hand, isolated bacterial species were found susceptible to antibiotics such as erythromycin, ciprofloxacin and gentamicin. Thus, suggesting that theseantibiotics have not been co mmonly used as the treatment of choice for mastitis in farms.

1788 - Establishing cross-FMT intervention for calf diarrhea treatment in pre-weaned calves

Author: Jahidul Islam, Natsuki Ohtani, Junya Yamazaki, Zen Nakamura, Hidekazu Tanaka, Tomonori Nochi

Objectives

Calf diarrhea is the most common illness in pre-weaned calves. Effective treatment options to manipulate the gut microbiome environment of calves under commercial operations are of great importance to improve animal health and reduce antimicrobial usage. Fecal microbiota transplantation (FMT) involves transplanting the fecal contents of a healthy donor into a diseased patient to restore gut microbiota to its original state. FMT has been demonstrated to be an effective treatment for calf diarrhea in recent times. However, most of the studies on FMT against calf diarrhea are investigated in a same farm specific manner concerning its safety. However, to introduce FMT as a nationwide positioning, as well as to better design future microbial therapeutics for calf diarrhea, we introduced cross- FMT intervention, in where donors were selected from three different regions within a range of approximately 3,000 km across Japan.

Material and methods

We collected 180 feces from healthy calves as donor from three regions of Japan (Hokkaido, Chiba, and Okinawa area, n=60 each), and among them 24 donors were finally selected as potential donors for FMT based on 16S rRNA amplicon sequencing and absence of potential pathogens especially *Clostridium perfringens*, *Cryptosporidium parvum*, *Coccidia*, *Salmonella*, *Rotavirus*, *Coronavirus*, *E. coli* as well as BLDV and BLV. From each potential donor, 3 vials (10 g feces each) were prepared using the original feces and continued to freeze-dry. Finally, prepared donors' feces were sent to three regions and subjected to 72 FMT trials (n=24 recipients/area).

Results

Based on the diarrheal severity, pathogen detection, 16S rRNA amplicon sequencing and fecal metabolomics (CE-TOFMS) results, overall FMT efficacy is found 76.39% 7 days after FMT. Based on the area, the FMT efficacy is observed in Hokkaido region (83.34%), Chiba (75.0%) and Okinawa (70.83%). Comparing the donor efficacy, the donor efficacy is observed in Hokkaido (76.67%), Chiba (77.78%) and Okinawa (73.34%). Finally, based on the intra-area donor efficacy, Hokkaido donor showed 100% efficacy in Hokkaido, but 80% for Chiba and 50% for Okinawa. On the contrary, Chiba donors showed similar efficacy (77.78%) in all three areas, while Okinawa donors showed 60% efficacy in Hokkaido and Chiba, but 100% efficacy in Okinawa itself. Fecal microbial community analysis based on 16S rRNA amplicon sequencing revealed an increase in the alpha-diversity indices after FMT and pairwise PERMANOVA analysis from beta diversity confirmed that there is significant difference before and after FMT in successful FMT cases. Furthermore, FMT reduces the gram negative *Proteobacteria* level as fecal amino acids (alanine, leucine, valine, isoleucine, glycine, arginine, and glutamic acid), which may be responsible to restore the gut microbial community to healthy state and lessens the diarrheal severity in successful cases.

Conclusions

Overall results confirmed that even though the donor-specific microbial consortia engraftment may occur in the recipients, based on the different area, their efficacy is varied. However, to transcend regional differences and improve the versatility of FMT donors, it is necessary to further investigate the global diversity in FMT donor-recipient relationships.

1789 - A preliminary necropsy- based study on bovine mortality in Italy

Author: Alessandro Necci, Paola Papa, Michele Tentellini, Federico Consalvi, Marco Gobbi, Silvia Crotti, Elisabetta Manuali, Nicoletta D'Avino

Objectives

In Italy, approximately 6 million cattle were raised in both 2019 and 2020, with a mortality rate of 4,6% in 2019 and 4,3% in 2020, as reported by the National Database Bank. However, no official data about causes of mortality are available. Although mortality rate is an important animal welfare index within the farm, understanding the cause of death for implementing resolution strategies is important too. Therefore, necropsy represents a relevant diagnostic tool in order to assist the veterinarians in differential diagnoses and the farmer in preventive management measures. To date, causes of cattle death in Italy based on complete necropsy diagnoses have not been summarized. The purpose of this retrospective study is to summarize the primary cause of mortality in all the cattle necropsied during 2019/2020 period at Istituto Zooprofilattico Sperimentale of Umbria and Marche region (IZSUM), one of the several official Diagnostic Laboratories in Italy.

Material and methods

Of all bovine necropsied at IZSUM from January 2019 to December 2020, 1067 were included in the study. Animals were submitted from the owners or the veterinarians to the laboratories, where history, age and attitude were recorded. Necropsies were performed by 4 veterinarians using standard procedures. On the basis of the macroscopic appearance, samples from pathologic organs were collected for further analyses (i.e. bacteriological, histopathologic, parasitological exams, bio-molecular and ELISA tests). Data recorded were entered into a database (Excel, Microsoft, Redmond, WA, USA) for further summary and analyses. First cause of death was determined based on the history (when available), gross and histopathologic lesions, and results of further exams. Data were elaborated with descriptive statistics analysis.

Results

In total 1067 bovine were necropsied from 2019 (53,5%) to 2020 (46,5%) at the IZSUM: 738 beef cows (68,6%), 328 dairy cows (30,5%) and 10 animals (0,9%) with unspecified aptitude due to lack of history. Animals originated from all over Italy, mainly from Umbria (422, 39,2%), Piedmont (156, 14,5%) and Marche (149, 13,8%) regions. They were categorized based on age: 420 calves between 1 and 30 days (C01; 39%), 235 calves between 1 month and 6 months (C02; 21,9%), 73 beef or heifer (C03; 6,8%), 129 cows (C04; 12%), 219 fetuses (C23,C24,C25 based on the age of pregnancy; 20,3%).Animals were also categorized based on the main syndrome found at necropsy: 457 gastrointestinal disease (42,5%), 252 respiratory disease (23,4%), 228 reproductive disease (21,2%), 10 cardiovascular syndrome (0,9%), 3 neurological syndrome (0,3%), 6 urogenital disease (0,5%), 4 musculoskeletal syndrome (0,4%), 1 mastitis (0,1%), 96 systemic syndrome (8,9%) and 19 trauma (1,8%). Primary cause of death was identified in 773 cases (71,8%). The most common one in C01 were gastrointestinal disease (59%), systemic syndrome (9%), respiratory disease (8,6%). No cause of death was evident in 81 cases (19,3%).Major causes of death in C02 were respiratory disease (50,2%), gastrointestinal disease (28,5%), systemic syndrome (3,4%) and trauma (1,7%), while no cause of mortality was identified in 38 cases (16,2%). Animals in C03 died more often of

respiratory disease (52,1 %) and gastrointestinal disease (17,8%), while no cause of death was evident in 12 cases (16,4%).The most common one in C04 were gastrointestinal disease (31,8%), respiratory disease (24,8%) and systemic syndrome (9,3%). No cause of death was identified in 30 cases (23,3%). Causes of abortion from fetuses were identified in 77 cases (35,2%).

Conclusions

Calves in C01 and C02 represent more than 60% of the animals necropsied in this study, as the most sensitive category within the farm. Gastrointestinal and respiratory disease were the most frequent causes of mortality among all animals necropsied (42,5 % and 23,4% respectively). Gastrointestinal disease represented the most important cause of mortality in C01, but it was relevant in C04 too. Instead, respiratory syndrome was more impactful in C02 and C03. These data agree with the authors experience and the updated literature. Authors believe that diagnosis only in 35,2% cases of fetuses and no diagnosis in 28,2% of the other cases were probably due to poor samples preservation or incomplete sampling or non-infectious causes. Further data will be analyzed to identify also the main etiological cause of death per each age category and disease.

1794 - Therapeutic efficacy of freeze-dried microbiota transplantation for calf diarrhea

Author: Natsuki Ohtani, Yu Shimizu, Jahidul Islam, Hidekazu Tanaka, Tomonori Nochi

Objectives
Fecal microbiota transplantation (FMT) has been proposed as a new treatment for calf diarrhea, but there are still many obstacles in establishing the procedure for fecal microbiota preparation. In this study, the microbiota was isolated from donor feces and subjected to lyophilization, a method commonly used to improve microbial stability. The therapeutic effect of freeze-dried microflora transplantation (FD-MT) on calf diarrhea was monitored by follow-up surveys before (D0), 1 day (D1), and 7 days (D7) after FD-MT.
Material and methods
A total of nine FD-MT was conducted on two farms in the same province. 9 healthy calves of Japanese black and F1 (Holstein Friesian and Japanese black) aged 18-61 days were used as donors, and 9 calves of Holstein Friesian, Japanese black and F1 (Holstein Friesian and Japanese black) aged 9-49 days with diarrhea were used as recipients. The freeze-dried microbiota prepared from 20 g of fresh donor feces were lyophilized in a freeze-dryer, and stored at -80℃ for about 2 months until the use for transplantation. FD-MT was conducted using a porcine uterine lavage catheter that was inserted into the rectum from the recipient's anus to a depth near the second lumbar vertebra (40-60 cm). From the recipients, fecal samples were collected on D0, D1 and D7, and blood samples were collected from the recipients on D0 and D7. The fecal and blood samples were used for investigating fecal score and fecal water content, for performing metagenome (MG), metabolome (MB) and blood biochemistry analyses, and for detecting pathogens as well as BLV and BVDV genes. The fresh feces collected from donors were analyzed for MG and MB analysis, and the freeze-dried microbiota of the donors were analyzed for MG analysis.
Results
There was no significant difference in donor microbiota composition between fresh and freeze-dried microbiota. The cure rate by FD-MT was 100% (9/9). After FD-MT, the fecal water content in the recipients decreased significantly. In contrast, total cholesterol level in serum increased significantly. The fecal microbiota gradually approximated to the donor freeze-dried microbiota in both two farms. Similarly, the fecal metabolites gradually approximated to the donor fresh feces in both two farms
Conclusions
FD-MT has proven to be as effective as conventional FMT in the treatment of diarrhea in calves, regardless of cause. The results suggest that freeze-drying is a useful method for preservation of fecal microbiota. This method allows for the preservation of donor feces of excellent quality, ensures safety and efficacy by pre-confirming the quality of donor feces and the presence of pathogens, allows for long-term stable supply to a large number of recipients, and simplifies a complex work process. The results of this study will help to solve issues in FMT therapy.
Keywords: Calf diarrhea, freeze-dry, microbiota, metagenomics, metabolomics.

1796 - STANDARDIZATION OF A COLORIMETRIC ISOTHERMAL AMPLIFICATION "LAMP" TECHNIQUE FOR DIAGNOSIS OF INFECTIOUS BOVINE RHINOTRACHEITIS.

Author: Fernando Cerón Téllez, Elizabeth Loza Rubio, Marisela Leal-Hernandez, Fernanda Juárez-Espinosa, Carlos Eduardo Aragón López

Objectives

Infectious Bovine Rhinotracheitis (IBR) is a contagious viral disease of cattle, belonging to the bovine respiratory complex. The clinical signs that characterize the disease are affection of the upper respiratory tract, and hyperemia of the snout This virus can also infect the genital tract and cause pustular vulvovaginitis, balanoposthitis, and abortions. In addition, it has a significant global presence and rapid dissemination; It is considered a veterinary public health problem due to its damage to animal production, so the diagnosis is essential. Currently, the Gold Standard for diagnosing IBR is PCR. [1] Isothermal amplification techniques have gained importance during the last 10 years since they are practical for use in the countryside. In 2000, Notomi et al. successfully established the Loop-mediated isothermal amplification (LAMP) method. In Mexico, IBR affects cattle producing severe economic losses. Although there are various techniques for its identification, implementing the LAMP technique would be very useful at the field level, since it only requires minimal equipment. Therefore, the goal of this study was to design and standardize the LAMP technique, to achieve the detection of IBR in samples of symptomatic cattle.

Material and methods

The bioinformatics design was carried out using NEB® LAMP PRIMER DESIGN TOOL for getting three pairs of primers using the US8 sequence gene that encodes the E glycoprotein of the Bovine α-Herpesvirus 1. For the positive control, we used Bovi-Shield GOLD® FP®5 VL5 vaccine, using an Invitrogen-Viral RNA / DNA Kit. After that, PCR was established and its amplification was verified using 1.5% agarose gel electrophoresis. For the first tests, 8 samples of bovine nasal and vaginal swabs were obtained, as well as an oropharyngeal swab of an aborted fetus from a center state of Mexican Republic. In order, to standardize the LAMP reaction, different concentrations of genetic material from the positive control were carried out by LAMP. Appropriate time and concentration were obtained.

Results

LAMP assay detected 15 positive and 2 negative samples; 9 samples were positive, both in LAMP and PCR. The average LAMP time for IBR virus is 30 minutes, similar to the Peltzer time reported in 2021 for the same pathogen [4]. However, in this trial, it is evident that in the nasal swab samples, using equal concentrations for both LAMP and PCR, LAMP could detect 5 samples that were not detected by PCR. Regarding the vaginal swab samples, these did present identical results using both techniques.

Conclusions

In this study, it was possible to standardize the LAMP technique in the laboratory and subsequently test it on some field samples. However, it is recommended to test more samples to be able to carry out an epidemiological study and validate it. These types of techniques would be very useful in mexican field, as screening the health of bovine populations, since the results are obtained in just 30 minutes. LAMP demonstrated

better results in terms of time and detection limit; However, it is reiterated that it is necessary to test more samples coming from different farms in order to be able to validate the results compared to reference techniques.

1798 - Molecular identification of bovine Coronavirus in respiratory problems in calves.

Author: Fernando Cerón Téllez, Marisela Leal-Hernandez, Jesus Zavaleta-Hernandez, Elizabeth Loza-Rubio, Marilu Bezares-Solis, Ana Gonzalez-Mendez

Objectives

Dairy cattle are the third economic activity of great economic relevance in Mexico; One of the main challenges in livestock farming is the economic losses caused by bovine respiratory syndrome and digestive problems. Among the primary agents are viruses; In recent years the coronavirus has been considered important. Diagnosis requires laboratory tests to confirm BoCoV, since recognition based on clinical and pathological elements is difficult due to its similarity to other diseases. Molecular diagnosis of BCoV is an important tool for disease control. Endpoint PCR is a rapid and sensitive technique that can be used to detect the genetic material of the virus. The objective of this work was the development and use of a PCR to detect a fragment of the gene that codes for protein S, in nasal exudate samples from calves with respiratory sinology

Material and methods

For the molecular detection of the virus, the reference search of the Mebus U0075.2 sequence was carried out. Subsequently, 100 bovine coronavirus sequences were analyzed, selecting a region of the S gene. To standardize the PCR procedure, a partial gene of the bovine coronavirus spike (S) protein was synthesized. The synthetic gene was constructed using the consensus sequence from the aligned sequences in Geneious Prime. The positive control was synthesized in T4OLIGO®, with the name CBovFCT; The synthetic gene is contained within a cloning vector called pEZclone-NRS-Amp-Blunt_HC, which maintains it in DNA form. The standardization of the PCR technique was carried out through DNA concentration and temperature tests.

Results

42 samples of calves with evident respiratory clinical sinology were analyzed, from which a nasal swab was performed for transfer to the INIFAP biotechnology laboratory. Afterwards, the RNA extraction was performed and the FAVORGEN® FavorPrep™ Viral Nucleic Acid Extraction Kit was used. I (with carrier RNA-low viral load) - FAVNK-001-2. The RNA was synthesized to complementary DNA which was used to amplify by PCR a 578bp fragment of the S gene, with the previously established protocol. Of the 42 samples, 10 positive samples were obtained; and the amplicons were purified and sent for sequencing. The sequences obtained were blasted and identified as bovine coronavirus sequences. In addition, a phylogenetic tree was created.

Conclusions

Through this work, the presence of bovine Coronavirus was demonstrated within the dairy production unit, which was found in nasal samples. It should be noted that there are few reports of BcoV, so it would be important to carry out a study with a larger number of samples to identify what type of genotypes are found in the country.

1802 - Staphylococcus aureus and methicillin-resistant staphylococci and mammaliicocci isolated from dairy cow milk in a high livestock density area of Northern Italy

Author: Sara Fusar Poli , Clara Locatelli, Valentina Monistero, Gustavo Freu, Laura Filippone Pavesi, Paola Cremonesi, Cristina Lecchi, Carla Maria Longueu, Sebastiana Tola, Alessandro Guaraglia, Maria Teresa Manfredi, Maria Filippa Addis

Objectives

Staphylococcus aureus is one of the most relevant causes of contagious mastitis in dairy cows, while non-*aureus* staphylococci and mammaliicocci (NASM), generally considered as minor pathogens, are among the species most frequently isolated from subclinical mastitis. In addition to potential udder health issues, NASM might constitute a reservoir for antimicrobial resistance (AMR) traits with potential for transfer to *S. aureus*. Furthermore, antibiotic treatment is frequently unsuccessful in the cure of staphylococcal infections, and this may also be related to antibiotic resistance. Specifically, methicillin-resistance is due to the presence of *mec*A or *mec*C gene and can be detected by phenotypic or genotypic methods. This is important because methicillin-resistant (MR) staphylococci are causes of human nosocomial and community infections, making them a relevant one-health issue. This study aimed to provide updated information on *S. aureus* prevalence in a European area with a high livestock density and to investigate MRSA presence and characteristics. Considering their potential role as resistance reservoirs and opportunistic pathogens, and the limited information available in the literature, we also investigated and characterized MR NASM.

Material and methods

A total of 88 samples of bulk tank milk (BTM) were collected from as many dairy farms located in Northern Italy. BTM was cultured on Baird Parker agar + rabbit plasma fibrinogen for the identification of *S. aureus* and on selective Chromogenic MRSA (CMR) for the detection of MR NASM. The identification of the investigated colonies was carried out through MALDI-TOF mass spectrometry. Then, 5 colonies identified as *S. aureus* on BPA and 1 colony identified as *Staphylococcus* spp. on CMR by MALDI-TOF MS were re-isolated for genotyping and molecular characterization. A Ribosomal Spacer PCR (RS-PCR) for typing and a qPCR for the investigation of the presence of genes involved in biofilm formation (*ica*A, *ica*D, *ica*B, *ica*C) were performed. The phenotypic confirmation of methicillin resistance for MR species was carried out by the disk diffusion method using an antibiotics panel to assess susceptibility to beta-lactams and other antibiotic classes. All *S. aureus* and presumptive MR isolates were tested for the presence of *mec*A and *mec*C genes by PCR and characterized for the SCCmec cassette.

Results

S. aureus was isolated in 32.95% (n = 29/88) of sampled farms with variable counts. All tested isolated carried the *ica* genes (*icaA*, *ica*B, *ica*C, *ica*D) indicating the potential to produce biofilm. Based on RS-PCR, *S. aureus* belonged to 10 different genotypes, with B being the most common (35.20%, n = 44/125 of isolates and 37.93%, n = 11/29 of positive farms). PCR performed on the 125 isolates detected the *mec*A gene in 8 samples, while the *mec*C gene was not detected. Upon enrichment and culture of BTM on CMR, 56.82% (n = 50/88) of the farms harbored MR staphylococci. Eight different MR

species were identified by MALDI-TOF MS. The most frequently identified MR species was *S. epidermidis* (MRSE, 35.59%) followed by *S. aureus* (MRSA, 18.64%), *M. sciuri* (15.25%), *S. haemolyticus* (MRSH, 13.56%), *S. saprophyticus* (11.86%), *S. cohnii*, *S. fleurettii,* and *S. pettenkoferi* (1.70% each). *mecA* was detected in 54 out of 59 MR isolates. None of the isolates was positive for *mecC*. Most MRSA carried type V SCC*mec* (45.45%), and one carried type IV (9.10%); the majority of MRSE carried type IV *SCCmec* (61.90%), followed by type II and V (9.52% each). Of 11 MRSA, five (45.45%) belonged to genotype S (GTS), two (18.18%) to GTBQ_I, and one each (0.91%) to GTAF_I, GTAT, GTQ, and GTZ. Numerous isolates were resistant to multiple antibiotics.

Conclusions

Our results suggest that the presence of MRSA and MR-NASM in dairy herds might be underestimated and provide new information on the prevalence and characteristics of circulating MR NASM that can be useful for future surveillance. Epidemiological monitoring of AMR in all staphylococci, including NASM, and stewardship programs for further reducing antibiotic use in dairy herds will be crucial for minimizing their diffusion in a one-health perspective.

1807 - Development of a multiplex PCR for the detection of mycoplasmas associated with reproductive disorders in cattle

Author: Anabelle Manzo Sandoval, Ana Silva González Méndez, Ana Victoria Zermeño Granados

Objectives

The objective of this work was to design and standardize a multiplex PCR for the identification of *Mycoplasma bovis*, *Mycoplasma bovigenitalium* and *Ureaplasma diversum*, microorganisms associated with reproductive problems in cattle.

Material and methods

From the gene sequence database [GenBank] of the National Center for Biotechnology Information (NCBI), the nucleotide sequences to *ureC* gene of *U. diversum*, *uvrC* gene of *M. bovis* and the 16S-23S intergenic spacer region of the ribosomal RNA (ITS 16S-23S rRNA) of *M. bovigenitalium*; from these, primers were designed and analyzed using [Primer 3 Plus], [Primer Designing Tool] from NCBI, [PrimerQuest Tool] from [Integrated DNA Technologies (IDT)] and [Geneious] softwares to amplify specific fragments of these sequences.

On the other hand, with [Geneious] software, a synthetic gene (gblock) consisting of the three sequences of the aforementioned fragments was designed to be used as a DNA template in the standardization of the multiplex PCR.

In order to establish the optimal alignment temperature for the three primer pairs, the reaction was submitted to a temperature gradient from 59°C to 63°C (with 0.5°C intervals). In addition, a $MgCl_2$ curve was made with concentrations of 1 mM, 1.25 mM, 1.5 mM and 1.75 mM to establish the ideal concentration to be used in the reaction.

PCR reactions were prepared using 1 µL of the gblock (0.079 ng/µL) and 14 µL of PCR mix, this consisted of 0.375 µL (250 mM) of each primer; 230 mM dNTPs mix; 0.62 units of Taq DNA Polymerase; 1X PCR buffer (proprietary formulation, pH 8.5), 1.5 mM $MgCl_2$ and sterile ultrapure water to a final volume of 15 µL. For the $MgCl_2$ curve, the concentrations indicated above were used. Amplification was performed with the following program: an initial step of 5 min at 95°C, followed by 35 cycles of 30 s/95°C, 30 s/59.5°C, 30 s/72°C and a final extension step of 5 min/72°C. In the case of the temperature gradient, it was necessary to modify the alignment temperature as previously noted. The amplified products were separated by 2% agarose gel electrophoresis, applying a voltage of 80 V for 60 min, subsequently the gel was stained with [SYBR Gold] following the manufacturer's instructions.

Results

Based on the bioinformatic analysis, 3 pairs of primers were designed and selected for the amplification of 325, 429 and 141 bp fragments from the *ureC* gene of *U. diversum*, *uvrC* of *M. bovis* and the ITS 16S-23S rRNA of *M. bovigenitalium*, respectively. On the other hand, the sequences for the construction of the gblock were ordered as follows: the sequence corresponding to the *uvrC* gene was placed at the 5' end, followed by the *ureC* gene and at the 3' end, the ITS 16S-23S rRNA region, resulting in a gblock of 905 bp, since it was necessary to include 5 bases in each one of the 3' and 5' ends.

It was determined that the temperature at which the three expected products amplify using this multiplex PCR is 59.5°C; whereas, at a concentration of 1.5 mM $MgCl_2$ these

amplified products are clearly visible. Assays are currently underway to determine the analytical sensitivity and specificity of this molecular test.

Conclusions

The PCR strategy described can be a useful tool both for the diagnosis of *U. diversum*, *M. bovis* and *M. bovigenitalium*, as well as to know the frequency of these mycoplasmas in cattle with reproductive problems.

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1811 - Bacteriological isolation and Loop-mediated isothermal amplification assay (LAMP) of Pasteurella multocida in calves

Author: Laura Hernandez Andrade, Israel Daniel Ricardo-González, Fernando Cerón-Tellez, Karla Rodríguez.Hernández, Xochitl Mendez-Amador

Objectives

The objective of the work was to isolate *Pasteurella multocida* and detect it using the LAMP test in calves with respiratory problems.

Material and methods

Nasal exudates were taken from 42 calves with respiratory problems to perform bacteriological isolation and molecular identification by LAMP. The samples were inoculated on blood and McConkey agar and incubated at 37 °C for 24 to 48 h in an aerobic atmosphere, the suspected *Pasteurella* colonies were identified by biochemical tests. In the LAMP reaction, WarmStart Colorimetric LAMP MasterMix with UDG was used, 5 ul of DNA from each sample was used and 3 ul of a *Pasteurella multocida* ATCC 12445 was used as a positive control. The positive control presented a colorimetric change according to the standard, the results were observed and were confirmed by electrophoresis. Endpoint PCR was also performed on the samples.

Results

The LAMP technique detected 15 positive and 27 negative samples, in the bacteriological isolation five strains of *Pasteurella multocida* were isolated. Using endpoint PCR, thirteen samples were positive and 29 were negative.

Conclusions

It is necessary to test a larger number of samples to confirm the sensitivity and specificity of LAMP. One of the advantages of the LAMP test is that it is easy to perform and takes little time.

1817 - Optimization of targeted enriched metagenomics protocol to obtain strain-level data for Mycoplasma bovis in respiratory samples from cattle

Author: Sal Lamsal , Christopher Panaretos, Ethan Dudley, Enrique Doster, Lee Pinnell, Matthew Scott, John Richeson, Hatem Kittana, Paul S. Morley, Robert Valeris-Chacin

Objectives

Bovine respiratory diseases (BRD) is a major health challenge to the beef cattle industry. *Mycoplasma bovis* has been linked to chronic cases of BRD. Advances in molecular techniques may provide further insights into *M. bovis* ecology, removing the bias associated with culture. The objective of this study was to develop and optimize a targeted enriched metagenomics protocol for *M. bovis* (using RNA baits to enrich *M. bovis* DNA) in samples from the respiratory tract of beef cattle. Specifically, we evaluated different molarity strengths of the baits because lower bait molarity may improve specific binding in conditions of low target abundance and drastically reduce the cost of the method. We also looked into the relative abundance of phylogenetic cluster sequence variant (PSV's) to tease out the strain level characterstics of *M. bovis.*

Material and methods

Thirty Nasopharyngeal swabs collected from West Texas A&M University feedlot were extracted using PowerSoil Pro (Qiagen) kit and were confirmed to be positive for *Mycoplasma bovis* using digital qPCR system.

These positive samples were used to optimize a targeted enriched metagenomics (TE) protocol. The bait set (n=29,296 baits) used for the TE protocol were designed using syotti and produced by Agilent. This bait set was aliquoted in four different molarities; quarter (Q), half (H), twoThirds (tTh), and full (F). All four molarities were tested in all samples. Two rounds of TE (double capture) and a pre-library preparation enrichment were used. DNA libraries were submitted to North Texas Genome Center for sequencing in an Illumina platform (NovaSeq).

Reads obtained from sequencing were aligned to the standard kraken2 database to calculate the number and percentage of reads on target i.e., classified as *M. bovis.* Reads classified as *M. bovis* were extracted using Kraken tools and re-classified using a custom Mycoplasmoidales database to obtain Phylogenetic Cluster Sequence Variant (PSV) level information on the reads.

DNA library concentration, percentage of target reads classified as M. bovis (with standard and custom database), percentage classified as PSV, and number of unique *M. bovis* PSV were compared between molarity strengths to determine the optimal concentration of baits. Generalized Estimating Equations (GEE) linear and logistic regression models were built using sample ID as the panel variable and an independent working correlation matrix. Margins were adjusted using Sidak method for multiple comparisons.

Results

Although we observed a linear decrease in the concentration of DNA in our final library, we did not see the significant effect of bait molarity in the classification of our targeted genome including species level classification and PSV's.Low concentration of bait molarity can be used to reduce the total cost of TE protocol.

Conclusions

Using targeted enriched metagenomics protocol to understand the strain level dynamics within the mycoplasma bovis species in cattle can help us understand the ecology of *M. bovis*.

1822 - Bubaline Genital Leptospirosis in females with reproductive failure. Preliminary results.

Author: Rosaura Pastora Pérez Gil, Carlos Linares

Objectives

This investigation aimed to use a bacteriological culture to demonstrate the presence of *Leptospira* spp. in the genital tract of Bubalus bubalis females from a herd of female buffaloes with low reproductive performance.

Material and methods

The analyses were performed on a farm in the state of Portuguesa in Venezuela. This farm reported 34 females out of 497 presenting abortions, birth deaths, weak offspring, low birth weight, poor maternal ability, and infecundity during the period Dec 2022 and Dec 2023. Thirty-four blood samples were extracted by puncture of the jugular vein, which was allowed to coagulate to obtain the serum. Animals were brought to a slaughterhouse, and the intact uteri with ovaries and ampullae from nine animals were collected. Cervical-uterine swabs, fresh tissue samples, and follicular fluid were taken for bacteriological culture, and a portion of the tissue sample was fixed in a 10% buffered formalin solution for histopathological analysis. The serum was evaluated with microscopic agglutination *test* (MAT) with a panel of seven leptospira serovars (*L.icterohaemorragiae*, *L.canicola, L.pomona, L.griphotyohosa, L.hardjo bovis, L hardjo pratjino* and *Bratislava*) individually. The culture of cervical-uterine swabs, fresh tissue samples, and follicular fluid was performed in tubes containing Ellinghausen–McCullough–Johnson–Harris medium (EMJH), a liquid culture medium selective for Leptospira.

Results

The rate of abortion in the head was 47%, occurring in average at day 207. The rate of stillbirth was 5.88%. The percentage of weak offspring was 11.76%, while the percentage of buffalo cows with poor maternal ability was 8.82%. In addition, the average of days open was 303 and the percentage of non-pregnant cows was 26.47%. The MAT results indicated titers of 1:50 to 1:800 for the *Icterohaemmorragiae* serovar in 94,1% of the samples; titers of 1:50 to 1:400 in serovars *Canicola* in 85%, *Pomona* 47,1% and *Grippotyphosa* in 55,9% of the samples. In the case of *Harjo bovis*, titers of 1:100 were detected in 5.9% of the samples, while *Harjo pratjino* in 2,9% and titers of 1:50 in 8,8% of the samples for the *Bratislava* serovar.There was growth of *Leptospira* spp. in 100% of the samples of the uterus, ovary, and oviduct, as well as in 100% of the cervical-uterine swab and 100% of the follicular fluid. The pathologic alterations found in the uterus were chronic non-suppurative atrophic endometritis (100% of samples). In oviduct: chronic salpingitis and fibroplasia 22.2%, edema 22.2%, atrophy and fibroplasia 11.1%. While in the ovary: polycystic and mature and persistent corpus luteum 33%, polycystic and atrophic 66%.

Conclusions

The serological titers show an active infection. The active serovar is I*cterohaemmorragiae* since it presents titers at a 1:800 dilution. The presence of the bacteria in uteri and oviducts with pathological alterations in the tissues generates a hostile environment for gametes and embryos and could be responsible for the poor reproductive performance of the herd. In addition, fetal infection prevents the adequate

growth of the fetus, which could explain the birth of weak, low-weight offspring and stillbirths and cause abortions. The growth of the bacteria in the follicular fluid can damage the oocyte, as well as the theca cells, and this could be related to the different ovarian pathologies observed. This evidence allows us to conclude that the animals suffered from Bovine Genital Leptospirosis (BGL).

1823 - Seroprevalence of Leptospira spp. in cattle farms in the state of Sonora, México, and risk factors that affect production.

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Objectives
Identify seroprevalence of *Leptospira* spp. in cattle from livestock farms in the state of Sonora, México, and risk factors that affect production.
Material and methods
Blood samples were taken from 460 cattle from 15 farms in the state of Sonora. Questionnaires were applied to know the productive and reproductive parameters of the farms included in the study. The sera were processed by the microagglutination test (MAT) with a panel of 13 *Leptospira* serovars. Total seroprevalence and per serovar were obtained. Subsequently, the effect of the presence of a natural serovar (*L.* hardjo) and an incidental serovar (*L.* bratislava) of *Leptospira* on the productive and reproductive parameters of cattle, as well as the serological concentrations of blood metabolites, was evaluated. Finally, risk factors that affect production were estimated, relating the serum values of these metabolites with the occurrence of reproductive problems in Sonora cattle.
Results
Results show 24.6% of the total sampled cattle and 93% of the farms were seropositive. In general, low productive and reproductive parameters were observed for the state of Sonora. It was found that females positive for *L.* bratislava had a higher percentage of abortions and repeated more estrus than those positive for *L.* hardjo ($p<0.05$). Alterations in blood metabolites were detected in the animals studied, which were mainly associated with malnutrition and dehydration processes. No statistical differences were found between metabolites and the presence of abortions in cows positive for *Leptospira* spp. However, the odds ratio (OR) analysis showed that low levels of AST play an important role as a risk factor for the presence of reproductive problems. While low or normal levels of BUN and hypermagnesemia turned out to be protective factors.
Conclusions
These data show us the current panorama of *Leptospira* spp. in cattle in the state of Sonora and its effect on productive and reproductive parameters. The presence of incidental serovars of *Leptospira* spp., such as *L.* bratislava, has a negative effect on the reproductive parameters of cattle in Sonora, but also malnutrition and dehydration, which shows that there are other factors that affect the evaluated indicators. On the other hand, the presence of incidental serovars reflects the need to limit the coexistence between different species in livestock production units and reinforce their surveillance. It is also important to consider that leptospirosis is an occupational risk zoonosis. Likewise, it is necessary to improve prevention, diagnosis, and therapeutic programs in Sonoran cattle.

PARASITIC DISEASES

1061 - Diversity, prevalence and burden of gastrointestinal parasites in the Hong Kong feral cattle and their associated risk factors

Author: Tania Ambre Perroux ., Samantha S. Y. Lie, Fraser I. Hill, Alan G. McElligott, George M. W. Hodgson, Winnie W. S. Wong, Kate J. Flay, Eloi Guarnieri

Objectives

Gastrointestinal parasites (GIP) are a common issue among ruminants, with extensive research dedicated to their impact on livestock. However, the study of GIP in wild or feral ruminants is less explored, leaving a gap in our understanding of parasite diversity, host susceptibility, and host-parasite interactions in these populations. This is particularly relevant in the 900 free-ranging feral cattle in Hong Kong, which do not receive anthelmintic intervention. The investigation of GIP in this specific population could provide valuable insights and inform future regulations of GIP management in wild populations. We aimed to:

8. Determine parasite diversity, prevalence and burden of GIP in these feral cattle
9. Identify associations between GIP prevalence, burden, and:
    b. Season
    c. Environment (herd and location)
    d. Individual characteristics (sex and body condition)

Material and methods

We sampled 175 adult cattle from 7 different herds in the dry (December 2022 to February 2023) and wet seasons (July to August 2023). 85 cattle were sampled in both seasons, 52 were collected only in the dry season and 36 only in the wet season. We collected 20-30g of freshly voided feces from each individual and assessed body condition score (BCS) based on a modified visual 9-point scale. We used McMaster's technique to determine the Fecal Egg Count (FEC) and Fecal Oocyst Count (FOC) of nematode eggs, cestode eggs and protozoan parasite oocysts, and used sedimentation to identify trematode eggs (Gibbons et al., 2022). Coproculture was used to identify nematode larvae (Van Wyk & Mayhew, 2013). Impact of season, environment and individual characteristics on GIP prevalence were analyzed using Generalized Linear Mixed-Models, and impact on GIP burden with Zero-Inflated Poisson models.

Results

Fifteen species of GIP were identified, comprising eleven species of nematodes (*Trichostrongylus* spp., *Cooperia* spp., *Ostertagia* spp., *Oesophagostomum* spp., *Trichuris* spp., *Dictyocaulus* spp., *Toxocara* spp., *Strongyloides* spp., *Haemonchus* spp., *Bunostomum* spp. and *Chabertia* spp.) and two trematode species (*Fasciola* spp. and *Paramphistomum* spp.). Only one protozoan (*Eimeria* spp.) and one cestode (*Moniezia* spp.) parasites were found.

FEC and overall prevalence of GIP in the Hong Kong feral cattle were much higher in the wet season (prevalence: 100%; mean FEC: 57.02) than in the dry season (prevalence: 97%; mean FEC: 14.89). Trematodes were more prevalent in smaller groups (92.86%)

than in larger ones (83%). Groups with no calves had higher prevalence of *Fasciola* spp. (17.41%) than groups with calves (14.29%). However, groups with more calves had higher prevalence of *Trichistrongylus* spp. (36.36% vs 11.44%), *Strongyle* spp. (35.71% vs 14.93%) and *Cooperia* spp. (24.24% vs 7.46%) than groups without calves.
Trichostrongylus spp. were three times more prevalent in males (24.35%) than females (8.84%). Males had higher overall FEC (33.48 vs 13.95), *Strongyles* spp. FEC (30.87 vs 12.59) and *Trichuris* spp. FEC (2.61 vs 1.36) than females. Females had higher *Eimeria* spp. FOC than males (155.10 vs 131.74). Nematode prevalence was lower in individuals with extreme BCS (BCS 4: 42.31% & BCS 7: 33.33%) than individuals with average BCS (BCS 5: 61.24% & BCS 6: 57%). Prevalence of *Strongyle* spp. decreased with an increase in body condition (BSC 4: 30.77% vs BCS 7: 0%). FEC decreased with increased BCS (BCS 4: 44.23 vs BCS 7: 8.33). *Eimeria* spp. oocyst egg counts increased with an increase in BCS (BCS 4: 53.85 vs BCS 7: 275).

Conclusions

Our research unearthed a strikingly high prevalence of GIP in a feral population devoid of routine care without significant clinical effects. This mirrors findings from studies conducted on free-range farmed cattle in tropical and subtropical regions. We also managed to pinpoint risk factors that significantly influence GIP prevalence and burden, including a substantial increase in GIP prevalence and burden in the wet season. However, the impact of these risk factors varied across parasite species, adding another layer of complexity to our understanding of GIP. This research presents an opportunity to measure the effect of GIP infection in feral cattle without anthelmintic intervention.

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Van Wyk, J. A., & Mayhew, E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. Onderstepoort Journal of Veterinary Research, 80(1), 1–14.

1080 - Estimating the prevalence of Giardia spp. on cattle farms in France from 3VCOCS data

Author: Tom Strydom

Objectives

This study was conducted to evaluate the prevalence of *Giardia* spp. in cattle faeces analyzed as part of the 3VCOCS service (a coproscopy service set up by MSD Animal Health France to diagnose the presence of parasites in young ruminants (< 6 months of age)). In addition to the diagnostic services by MSD Animal Health, 3 analytical methods were compared for diagnosis of giardiasis. Giardiasis is a widespread protozoosis in Europe, both in humans and animals. In ruminants, it causes growth retardation, possibly accompanied by intermittent diarrhea. In France, testing for *Giardia* spp. is not common, and its prevalence on cattle farms is poorly understood. A previous study in 2016 estimated that on 77.3% of cattle farms, at least one calf was infested with the parasite.

Material and methods

From September to December 2022, 143 cattle farms submitted fecal samples from calves to the laboratory as part of the 3VCOCS service. The 635 faecal samples were analyzed using a direct microscopic examination of each sample with Lugol staining, a direct microscopic examination of the pooled faeces with Lugol staining, and the modified Ritchie's method. For the modified Ritchie's method 1 gram of feces is diluted in 5mL of chlorhydric acid 15%, filtrated through a gauze cloth, mixed with an equivalent volume of ether and then centrifugated. The supernatant is then removed, and one drop of the sediment is placed on a microscopic slide with one drop of Lugol. For this study, the age category, the presence of *Eimeria* and the individual appearance of the faeces (liquid or molded) were recorded for each farm.

Results

Of the 143 farms, 37 were suckler farms, 77 were dairy farms and 29 farms did not report their type of production system. Prevalence by farm type was different regarding the production (beef or dairy) and the coproscopy method. For dairy farms, the prevalence was 31.2%, 20.8% and 13.2% with the individual Lugol method, the pooled Lugol method and the Modified Ritchie's Method respectively. For beef farms, the prevalence was 16.2%, 10.8% and 10.8% with the individual Lugol method, the pooled Lugol method and the Modified Ritchie's Method respectively. From the results, *Giardia* spp. was twice as present on dairy farms compared to beef farms.

The prevalence of faecal excretion of *Giardia* spp. in trophozoite form was 19.4% among all samples (method of reference: individual Lugol method). The sensitivity and specificity of the pooled Lugol method for the detection of trophozoites was 58.6% and 99.1%, respectively. The Positive Predictive Value (PPV) of the Lugol pooled method for the detection of trophozoites was therefore 94.0% and the Negative Predictive Value (NPV) was 90.9%. The modified Ritchie's method only detects *Giardia* spp. cysts, the prevalence thereof was 26.3% among all farms. Compared to the modified Ritchie's method, the sensitivity and specificity of the individual Lugol method for cyst detection was 57.1% and 100%, respectively. The sensitivity and specificity of the pooled Lugol method for cyst detection was 11.4% and 100%, respectively.

There was no correlation between the faecal score and the presence of cysts. However, there was a strong correlation between the fecal score and the presence of trophozoites($p<0.001$). This can be explained by the fact that trophozoites are the form of multiplication of *Giardia* spp. in the digestive tract and that, during giardiasis, especially during diarrheal episodes, they can be excreted with faeces. Trophozoites were found in 27% of liquid faeces (n = 36/133), compared to 1.4% found in “molded” faeces (n = 7/502). There was a high correlation between the presence of high *Eimeria* spp. counts and the presence of liquid stools. The age of the animals had no influence on the rate of infestation by *Giardia* spp.

Conclusions

From the samples received, *Giardia* spp. was detected on 25% of the farms in its trophozoite or cystic form. The prevalence of the parasite seemed to be higher on dairy farms than beef farms. The prevalence of *Giardia* spp. was found to be 4 times more on dairy farms (n=16/77) than suckler farms (n=2/37) from the sample received. Of the three methods, the modified Ritchie's method allowed better detection of cysts but is not indicated to detect trophozoites. The trophozoite form is more often detected in the diarrheal feces. The pooled Lugol method is less sensitive than the individual analysis. Increasing the number of samples over the time could improve the sensitivity of the pooled Lugol method.

1084 - Old molecules new focus. Combining Ivermectin, Clorsulon, and Nitroxynil to combat Ivermectin-resistant barber's pole worms (Haemonchus spp.) in cattle.

Author: Matthew BALL, Luc DUREL , Louisa Van Wyk

Objectives

A range of helminth species often infests livestock, the most significant being gastrointestinal nematodes (GIN), lungworms, and liver flukes. These parasites can lead to severe illnesses and are among the most crucial diseases limiting grazing ruminants' production globally. Australian livestock, predominantly based on pasture all year round, do not escape from this and are heavily burdened by internal parasites. For decades, helminth infestations have been controlled through various categories of anthelmintics, especially macrocyclic lactones (ML). However, farmers and veterinarians worldwide are now encountering treatment failures due to anthelmintic resistance, similar to the issue of antimicrobial resistance. Among roundworms, *Haemonchus* spp. or barber's pole worm (BPW) express multidrug resistance. The objective of this study was to investigate the level of resistance to avermectins and moxidectin in livestock nematodes and to demonstrate the effectiveness of an ivermectin, clorsulon, and nitroxynil combination (primarily used for liver fluke control) to also control ivermectin-resistant BPW infection in cattle.

Material and methods

At the Department of Primary Industries of New South Wales initiative and Local Land Service of Tablelands, 250 young cattle were purchased on 71 properties and came into an experimental government farm. The cattle had been exposed to spontaneous parasitic infestations on their property of origin. The worm egg count (WEC) and BPW egg count (BPWEC) estimated the parasitic infestation. In pre-treatment (D-14), animals were tested, and the population was stratified based on WEC. Animals were then randomly allocated to have equal mean WEC per group. Besides the control group (B), six treatment groups were defined: Ivermectin 1% (G), Ivermectin 1% plus clorsulon 10% (D), Doramectin 1% (F), Moxidectin 1% (E), Moxidectin 10% (C), and Ivermectin 0.67% plus Clorsulon 6.7% plus Nitroxynil 34% (A). All treatments were given by injection on D0. Animals were then tested on D14, D28, D42 and D63.

Results

In pre-treatment, coproscopic analysis revealed that the parasitic population was made on 61% *Cooperia* spp., 36% *Haemonchus* spp., *Ostertagia,* and *Oesophagostomun* spp. for the remaining species. In animals treated with ML as single active or combined with clorsulon (C,E,F,G & D), WEC declined by 32, 46, 75, and 83%, respectively, from D0 to D14. WEC reduction was also observed in the A group (89%) and the control group (16%). In all groups, WEC increased by D28 and then progressively declined. By D63, WEC reduction vs D0 was 75,85,63,36 and 68% for groups C,E,F,G, and D, respectively. Regarding BPW egg count, reduction >95% was observed by D14 with an application of Moxidectin 1% (group E, 99%), Moxidectin 10% (group C, 100%), and Nitroxynil/Clorsulon/Ivermectin (group A, 100%). BPWEC slightly increased on D28 in all groups but group A (100%). By D63, the residual BPWEC was 83% for Moxidectin 1% (E), 94% for Moxidectin 10% (C), and 87% for the combined solution of Nitroxynil

Conclusions

This study confirms that avermectin resistance is widespread in Australia and easily noticeable by cattle producers. The predominance of *Cooperia* spp. and the high level of resistance to anthelmintics in this group compromises the effectiveness of all macrocyclic lactones, including Moxidectin. The combination of Nitroxynil/Clorsulon/Ivermectin was highly effective against ivermectin-resistant *Haemonchus* spp. with an efficacy of 100% at D14, identical to the efficacy observed with 10% Moxidectin. Using a Nitroxynil-containing formulation, with a mechanism of action different from that of the MLs, will likely help to prolong the effective use of moxidectin for *Haemonchus* control in Australian animal health programs.

1086 - Efficacy of an injectable combination of Nitroxynil, Clorsulon and Ivermectin against triclabendazole-resistant liver fluke (Fasciola hepatica)

Author: Matthew BALL, Luc DUREL , Louisa Van Wyk

Objectives

Livestock are often plagued by various helminth species, the most significant of which are gastrointestinal nematodes, lungworms, and liver flukes (LF). Australian livestock, which primarily rely on pasture year-round are heavily burdened by internal parasites. Studies have identified multi-drug resistance (avermectin, benzimidazole, and levamisole) in helminths on a high proportion of farms, and resistance to liver fluke treatments has also been identified in cattle farms in Australia. Mainly, resistance to triclabendazole (TBZ) is concerning and must be seriously considered. The injectable drug Nitroxynil is classically recommended for controlling multiple parasites at the standard average dose of 10 mg/kg BW, subcutaneously, including late development stages of LF. It is also known that Nitroxynil and Clorsulon act synergistically in both adult and immature stages of the parasite. This study evaluated the effectiveness of an Ivermectin, Clorsulon, and Nitroxynil combination to control triclabendazole-resistant *F. hepatica* infection in cattle.

Material and methods

Forty-two young female cattle were purchased and transported to an experimental station. Twenty days (D-20) prior to beginning the study, the animals were given a mixture of triclabendazole and levamisole and stratified based on their weight to form seven groups with similar characteristics. Two days (D-2) before the start of the trial, animals were tested (fecal egg count and egg identification) and regarded as free of *F. hepatica* infestation. On D0, the cattle were weighed and infested with approximately 430 metacercariae of *F. hepatica* from an Australian strain resistant to triclabendazole. The study's endpoints were the total number of live flukes in the liver, numbered at the slaughterhouse (D98), and the fecal egg count (FEC) tested every week, from D70 to the end (D77, D84, D91, and D98). Animals were allocated to a treatment group and dosed on D14, D28 or D84. One group was used as a negative control (CTRL). Two groups of animals were treated on the same day but with a different medicine. On D14, two groups of animals were treated with either Nitroxynil/Clorsulon (NTX/CLO) or Nitroxynil/Clorsulon/ivermectin (NTX/CLO/IVM) by injection. On D28, two other groups were given TBZ or NTX/CLO. On D84, the two remaining groups were treated with NTX/CLO or NTX/CLO/IVM.

Results

As expected, the TBZ treatment did not control the disease. Geometric mean live liver fluke counts were 82.4 and 129.3 in CTRL and TBZ-treated animals, respectively. Treatments given 14 days after infestation significantly reduced parasitism, but the reduction of the parasitic load never achieved more than 95%, with a residual infestation of 5 and 7.7 live flukes for NTX/CLO and NTX/CLO/IVM groups, respectively. Treatments combining NTX/CLO (with or without IVM) were found to be particularly effective when given 28 days after infestation for the immature stages and 84 days on adult flukes, with geometric mean fluke counts below 0.5, >99% reduction of adult fluke in the liver.

As expected, FEC constantly increased in the control and TBZ-treated groups over the study period. Interestingly, egg shedding sharply dropped in NTX/CLO and NTX/CLO/IVM-treated animals when treated on D84 compared to TBZ and controls. When the same drugs were given on D14, FEC slowly increased until D84, then plateaued. When NTX/CLO was administered on D28, FEC remained null until the study's end.

Conclusions

In cattle exposed to triclabendazole-resistant Fasciola hepatica, a combination of Nitroxynil and Clorsulon and/or ivermectin can achieve more than 99% reduction of the liver infestation when applied to animals infested by adult flukes or 28-days larva.

1119 - Two clinical studies to evaluate the efficacy of a novel subunit vaccine in the reduction of clinical signs (i.e. diarrhea) caused by C. parvum infections in neonatal calves

Author: Geert Vertenten, Martine Reijnders, Mark van Roosmalen, Hans Holtslag, Susan Arts, Suzanne Pel, Divine Dufé, Marianne Kaashoek, Christophe Roy, Nicolas Herman, Alfredo Teixeira

Objectives

Neonatal Calf Diarrhea (NCD) leads to calf mortality in cattle industries worldwide. *Cryptosporidium parvum* (*C. parvum*) causes a large proportion of these NCD cases.

A recent discovery of a glycopeptide (GP) epitope on the *Cryptosporidium* parasite recognized by a monoclonal antibody has led to the development of the GP40 subunit vaccine (Bhalchandra, 2023). When future dams are vaccinated with this vaccine, they generate antibodies against Gp40 and produce antibody-enriched colostrum which, when consumed by newborn calves, reduces clinical signs, such as diarrhea, caused by *C. parvum*. This vaccine [Bovilis Cryptium®] has shown its safety and efficacy in pre-clinical studies. To evaluate its effectiveness in animals in the field, two clinical studies were conducted.

Material and methods

Two multisite, randomized, negative controlled and double-blind clinical studies were conducted to evaluate the efficacy of this vaccine in the prevention of *C. parvum* caused diarrhea in dairy and suckling calves. Study authorisations were obtained and following farm screening 8 dairy farms in the Netherlands and 8 beef farms in France were selected for inclusion. All farms had a confirmed history of cryptosporidiosis in young calves. The animals vaccinated in these studies were healthy cows and heifers between 10 and 6 weeks before the expected date of calving. Study treatment consisted of subcutaneous application of one dose of [Bovilis Cryptium] vaccine in the neck for the test group, and saline solution in the control group. The treatment was repeated after 4 weeks. Animals were randomly assigned to the study groups 1:1, stratified by site. Calves, born from the study dams were automatically included in the study, but excluded from data analysis in case they received colostrum from any other than their own dam. All dams in the study were concurrently vaccinated with [Bovilis® Rotavec® Corona] to minimize the effect of other NCD causes. Dairy calves were fed with at least 3 litres of colostrum within 4 hours after birth. On the subsequent days at least 1 (day 1) and 0.5 (day 2-4) litres were fed. In the suckling herds, calves were allowed to suckle their dam ad libitum for at least the first 4 days of life.

The purpose of these studies was to detect reduction of the incidence, severity, and duration of diarrhea in calves drinking colostrum from vaccinated dams. Additionally, the general health and number of treatments against diarrhea was compared between the study groups and to confirm the antibody response to vaccination, colostrum samples of all dams were tested for antibodies against the Gp40 protein of *C. parvum*. Passive transfer of antibodies was tested by analysing the calves' serum after colostrum intake. To assess these parameters, all calves were scored for general health, feed intake and fecal consistency (Fc)* daily during 21 days after birth.

Results

The protective effect of vaccination was shown in the odds ratios for the efficacy parameters in both independent studies. These were in favour of the calves from vaccinated dams. In the dairy study, the calves from vaccinated dams had 23% less chance of diarrhea (Fc score ≥2), and in case of diarrhea, they had 34% less chance of severe diarrhea (Fc scores ≥ 3) and 26% less chance of having *Cryptosporidium* in their feces. The calves from the test group also had 24% less chance of scoring >0 (not healthy) in the Total Clinical Score (TCS = sum of the daily faecal consistency, general health and feed intake scores), as well as 26% less chance of requiring treatment against diarrhea. Additionally, the average duration of the diarrhea episodes was significantly reduced from 2.2 days in the control group to 1.6 days in the test group (Mixed model ANOVA p=0.0300).

Even though in the farms of the suckling calves the incidence of diarrhea was much lower than anticipated, also in this study the same parameter scores all showed lower trends in the calves of the vaccinated groups.

Additionally, in both studies the levels of antibodies against Gp40 were markedly higher in colostrum as well as calves' serum in the vaccinated groups compared to the control groups.

Conclusions

Overall, these results confirm that this newly developed vaccine is efficacious in mitigating the impact of *C. parvum* caused diarrhea in neonatal calves.

1120 - Efficacy of a Fluazuron, Chlorpyrifos, Cypermethrin, and Piperonyl butoxide combination in controlling the cattle tick (Rhipicephalus microplus) in moist tropical areas of Mexico

Author: Eliseo VELAZCO, Bruno SIVIERI DE LIMA, Mariel AGUILAR DOMINGUEZ

Objectives

Ticks harm more than 75% of the world's bovine population, and Mexico is no exception. The frequent use of acaricides has led to the selection of resistant cattle tick (*Rhipicephalus microplus*) populations. Given the increasing ineffectiveness of major chemical compounds for tick control, some researchers have suggested using combinations of active ingredients to seek synergism between them and overcome resistance to acaricides. The objective was to assess the efficacy of topical application of a product based on 2.5% Fluazuron, 7% Chlorpyrifos, 6% Cypermethrin, and 6% Piperonil butoxide in doses of 1 ml/10 kg of body weight for the control of natural cattle tick infestations in the moist Mexican tropics.

Material and methods

This study was conducted in moist tropical southeastern Mexico, where biotic conditions allow cattle tick populations to thrive throughout the year. Forty Simbrah beef cows with calves were exempted from parasite control for 21 days before the start of the study. The animals were kept on pasture and became naturally infested by the cattle tick, an endemic parasite of this area. On day zero, groups were stratified based on the average number of engorged ticks (Ø ≧ 4.0 mm) quantified on day -1, before 9:00 am. The cows were randomly divided into the control group (C, n=20 animals) and the experimental group (T, n=20). The experimental group received a dose of a commercial acaricide by a pour-on application at the rate of 1ml/10kg (FORTIK®4, Virbac, Mexico), while the Control group received a saline solution. The number of adult ticks was quantified on days 3, 7, 14, 28, and 35 after treatment. All animals were inspected for any related adverse reactions on the day of treatment and during measurements. The number of ticks on the animals in groups C and T was compared using Student's *t*-test and was considered significantly different when $p<0.05$.

Results

During the test, cattle in both groups carried ticks. On day -1 and day 3, there were no significant differences between treatment groups. However, a significantly ($P<0.01$) lower number of ticks was observed on treated animals from day 7 (28.5 ± 4 vs. 79±14.3 ticks, for group T and C, respectively) to day 35 (57.2 ± 14.7 vs 304.9±42.8 ticks), with a nadir on day 21 (4.2 ± 0.9 vs. 50.3±12.3 ticks). Tick counts were not collected after day 35, as planned.

Conclusions

As mentioned by others, the product's combination of acaricide substances has a higher toxic effect on the cattle tick than the ingredients used independently. It is difficult for a single-active topical acaricide to achieve such results in the context of *Rh.microplus*'s resistance to acaricides. Therefore, drugs should be combined to limit the erosion of the efficacy of each active substance. A periodic assessment of the sensitivity of the parasite to the different active ingredients and their combination should be carried out in each livestock operation. Investigations should ensure a balance between animal health and safety as well as environmental safety.

1125 - Milk production in dairy cows with natural infection by nematodes, after treatment with injectable 2% eprinomectin in the peripartum

Author: Carlos Fernandes Carvalho, Ana Cristina Figueiredo, Lucas Dias, Tales Cordeiro, Marcos Maçacco, Rogerio Rossi, Pedro Pinho, Damien Achard

Objectives

The objective of this study was to evaluate the effect of one administration of a 2% injectable eprinomectin on milk production in peripartum dairy cows naturally infected by gastrointestinal nematodes.

Thanks: Ceva Animal Health, Biotran - Brazil.

Material and methods

One hundred and ninety two (192) healthy and pregnant dairy females, from five different herds, managed under extensive conditions (pasture), not treated with endectocides in the last 120 days or with specific anthelmintics in the last 60 days, were randomly allocated according to their parity into two groups: A (N=96) injectable 2% Eprinomectin - 1 mL/100 kg body weight SC (200 mcg/kg body weight) and B (N=96) 1 mL/100 kg body weight of the placebo of the commercial product, SC. The treatments were given in the peripartum period (between a maximum of 7 days before and a maximum of 7 days after calving). The products were packaged in identical bottles only labeled as product A or B (blind study). Every week, from drying off until seventh week postpartum, individual fecal samples were collected, and fecal egg counts (FEC) were performed. Individual fecal samples collected weekly and by treatment group, were homogenized and then subjected to coprocultures with subsequent identification of the genus of nematodes obtained. Daily milk production was measured weekly from the day of calving up to the seventh week postpartum. The evaluation of the body condition score (BCS) was carried out weekly, at the time of feces collection, using scale 1 to 5 (EDMONSON et al., 1989). The results of parasitological exams and milk production were compared between the groups (intergroup assessment). And within the same group (intragroup assessment), at different collection times. All tests were considered at a 5% significance level using the SAS Studio.

Results

There were no adverse events during the study. There was a significant difference ($P<0.05$) in the mean EPG counts between the two groups in the week of calving and in the two subsequent weeks. No difference ($P>0.05$) was observed in the occurrence of any of the species of larvae evaluated between the groups at the different evaluation times. No difference ($P>0.05$) was observed in the BCS averages between the groups at any of the evaluation moments. In contrast, milk production was affected by treatment. During the study, treated cows produced more milk (20.6±5.2 vs 19.4±5.5 kg - $P=0.0002$) than control group. Analyzing the postpartum weeks separately, in the second and third week postpartum cows in group treated cows produced more milk (19.7±4.1 Kg/day vs 19.1±3.9 Kg/day and 20.2±4.8 vs 19.3±4.6 Kg/day, respectively - $P<0.05$), Additionally, there was a tendency ($P<0,1$) for greater milk production in cows in the treated group in weeks 4 to 7.

Conclusions

It is concluded that the treatment of bovine females in the week of parturition with 1 mL/100 kg body weight of Eprecis®, corresponding to 200 mcg of eprinomectin/Kg body

weight, subcutaneously, in a single dose, is safe and improves postpartum milk production.

1131 - ASSESSMENT OF THE SEROLOGICAL ANTI-GP40 TITERS OF NON-VACCINATED BEEF CATTLE BEFORE AND AFTER CALVING IN FRANCE.

Author: Clara Bourel, Geert Vertenten ., Thibault Jozan, Geert Vertenten, Andre Preto

Objectives

Cryptosporidiosis, caused by *Cryptosporidium parvum*, is a severe disease of young calves resulting in diarrhoea, loss of weight and sometimes in mortality. *Cryptosporidium parvum* is detected in about 60% of the faecal samples of diarrheic calves in France (Ninio, 2017). The disease has been controlled until now either with halofuginone or with paromomycin (antibiotic only for curative purpose). A new vaccine against cryptosporidiosis (Bovilis® Cryptium®) is now available in France to control the disease. The active component of the vaccine is the glycoprotein 40 (Gp40), a surface antigen of the parasite.

The study aimed to assess the serological anti-Gp40 titers of non-vaccinated beef cattle before and after calving in France.

Material and methods

Sixteen farms, representative of the French beef cattle production, were included. These farms participated to a larger study on respiratory diseases with regular sampling. The serological anti-Gp40 titers of the animals were analysed at the same time as the other serological analysis necessary for the respiratory study. From these 16 farms, 8 farms had performed a first sampling before calving. For the 8 other farms, cows had already calved before the first sampling.

Pregnant heifers and cows of the farms were sampled three times: Once when returning definitively to the stable (in autumn), a second time one or two months later and the third time, before the release to pasture in the following year (in spring).

Blood samples were shipped to the Center for Diagnostic Solutions (MSD Animal Health, Boxmeer, The Netherlands). An ELISA Gp40 was performed for each sample.

Results

In total, 236 cows (37 heifers, 199 multiparous) from 16 farms were included in this study. From these 236 cows, 148 cows were sampled three times. One or two samples were missing for the remaining 88 cows. A total of 610 blood samples were analysed.

The average time between first sampling and calving for the 54 cows sampled before calving (from 8 farms only) was 35 days, with a minimum of 2 days and a maximum of 79 days. The Gp40 titer didn't decrease when samples were taken closer to calving, possibly by the low number of samples measured.

From the 610 samples, 81 samples were taken before calving. From these 81 samples, anti-Gp40 titers varied from 9(log2) to 14.6(log2) with an average of 11.1(log2). There was no significant difference between the farms. There was no difference between the average in anti-Gp40 titer of pregnant heifers (10.9(log2) ; n 20 ; min 9.8 ; max 13.4) and pregnant multiparous cows (11.2(log2) ; n 61 ; min 9.0 ; max 14.6).The anti-Gp40 titer after calving (526 samples) varied from 8.8(log2) to 19.9(log2) with an average of 11.0(log2). There was no significant difference between the farms neither between before or after calving. No difference was observed between the average anti-Gp40 titer of calved heifers (10.9(log2) ; n 66 ; min 8.8 ; max 13.7) and calved multiparous cows (11.0(log2) ; n 460 ; min 8.8 ; max 19.9).

The Gp40 titer remained stable over the period (autumn 2022 to spring 2023).

Conclusions

The serum Gp40 titer of beef cows were stable over the period of calving. These titers were equivalent between the farms. The pregnant heifers and multiparous cows had the same titers before and after calving.

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C. Ninio, K. Pecceu, A. Dupont. Actualisation sur la prévalence terrain des principaux agents de diarrhées néonatales des veaux en France, Recueil des conférences, JNGTV 2017

1137 - Efficacy of a new fixed-dose combination injectable (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) in Australian cattle against artificial infections of gastrointestinal nematodes

Author: Raj Packianathan, Frederico Moreira , Andrew Hodge, Jacqueline Wright, Michael Pearce, Andrew DeRosa, Jase Ball

Objectives

We describe a new fixed-dose combination injectable (FDCI) formulated with doramectin and levamisole hydrochloride (HCl) to target broad and overlapping spectra of gastrointestinal nematodes (GINs) through two distinct modes of action. Here, we demonstrate the superior efficacy of the FDCI against mixed populations of cattle GINs in two dose confirmation studies conducted in Australia using artificially induced adult (Study 1) and immature (Study 2) GIN infections.

Material and methods

Artificial infections consisted of *Cooperia* spp., *Haemonchus placei*, *Ostertagia ostertagi* and *Trichostrongylus axei*. In both studies, cattle were inoculated with third-stage larvae and infections were confirmed by fecal egg count (FEC). Treatment groups in both studies were as follows: (1) negative control (saline, 0.9% sodium chloride), (2) positive control injectable endectocide (Study 1 - 0.2 mg.kg ivermectin; Study 2 - 0.2 mg.kg doramectin), (3) positive control injectable anthelmintic (7.5 mg/kg levamisole HCl) and (4) FDCI (0.2 mg/kg doramectin + 6.0 mg/kg levamisole HCl). Cattle were treated either 28 days (Study 1) or 6 days (Study 2) post-infection. On Days 14 to 16 (Study 1) or Days 20 to 21 (Study 2) post-treatment, cattle were euthanized and necropsied for the recovery, identification, and enumeration of worms. Treatment efficacy was calculated as reduction in worm burdens of treated cattle compared to saline-treated cattle, and treatments were considered effective if the geometric mean worm burden in the treatment group was reduced by ≥ 95% compared to the negative control group.

Results

In both studies, saline-treated cattle remained positive for GIN infections for the study duration. Ivermectin was less than 95% effective against *Cooperia* spp. (80.2%) and *H. placei* (24.8%) in Study 1, and levamisole HCl was less than 95% effective against *Ostertagia* spp. (47.1%) in Study 2. In contrast, the novel FDCI was 100% effective in treating for adult and immature life stages of all cattle GINs included in the artificial infections, with no worms recovered at necropsy from doramectin + levamisole HCl-treated cattle.

Conclusions

These data show a single administration of the FDCI provides broad-spectrum treatment of economically important cattle GINs.

1138 - Efficacy of a fixed-dose combination injectable (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) in Australian cattle against naturally acquired gastrointestinal nematode infections

Author: Raj Packianathan, Frederico Moreira , Andrew Hodge, Jacqueline Wright, Michael Pearce, Andrew DeRosa, Jase Ball

Objectives

Australian producers have long used macrocyclic lactones (MLs) to successfully control cattle gastrointestinal nematodes (GINs) and consequently improve production parameters. However, the trajectory of ML resistance development in cattle GINs is following that of small ruminant nematode populations, highlighting a need for novel treatment options to provide efficacy in the current environment and interrupt the long-term establishment of ML-resistant GIN populations in Australian cattle. Here, we describe three field studies conducted in Australia to evaluate the efficacy of a single administration of a novel fixed-dose combination injectable (FDCI) endectocide against naturally acquired infections of cattle GINs.

Material and methods

The FDCI is administered subcutaneously to deliver 0.2 mg/kg doramectin and 6 mg/kg levamisole hydrochloride (HCl). Study sites consisted of three farms in New South Wales (n = 2) and Victoria (n = 1). At each site, cattle were randomly allocated into one of three treatment groups: (1) untreated control (saline), (2) FDCI (0.2 mg/kg doramectin, 6 mg/kg levamisole HCl) or (3) positive control (0.2 mg/kg ivermectin). All treatments were administered on Day 0. Fecal samples were collected prior to treatment on Days -1 (Study 3) or 0 (Studies 1 and 2) and again on Day 14 (post-treatment) to evaluate efficacy via fecal egg count (FEC) and for coproculture.

Results

Adequacy of infection was confirmed at all three study sites, with Day 14 geometric mean (GM) FECs for saline-treated cattle ranging from 32.5 eggs per gram (EPG) to 623.7 EPG. FECs for FDCI-treated cattle were significantly reduced compared to saline-treated cattle ($p \leq 0.0001$) on Day 14, with GM-based efficacy ≥ 99.7% at all three study sites. In contrast, ivermectin was 97.4% effective against cattle GINs in Study 1 but was only 47.2% and 39.8% effective at study site 2 and 3, respectively. Genus-specific efficacies suggest the presence of ivermectin-resistant *Cooperia* spp. (Study 1), *Haemonchus* spp. (Study 2) and *Ostertagia* spp. (Study 3) populations in the naturally infected cattle used in these studies.

Conclusions

The post-treatment FEC and genus-specific efficacy estimations indicate the doramectin + levamisole HCl FDCI was highly efficacious against cattle GINs even in the face of ivermectin LOE at study sites 2 and 3. The efficacy of the new FDCI against both ML-susceptible and ML-resistant economically important cattle GINs in Australia affirms it is a valuable treatment option for producers operating in an environment of ML loss of efficacy.

1166 - Efficacy of EXZOLT 5% Pour-On against Rhipicephalus microplus ticks on two beef farms in Argentina

Author: Fernando Fader, Tom Strydom , Facundo Picolini, Eduardo Lopez, Guillermo Gargantini

Objectives

To demonstrate the efficacy of a fluralaner pour-on formulation (Exzolt 5% Pour-on) after its administration to cattle infested with *Rhipicephalus microplus* ticks resistant to commonly used ectoparasiticides under field conditions.

Material and methods

Two beef farms with established population of ticks resistant to commonly used ectoparasiticides were selected by organizations such as INTA, FUCOFA, FUCOSA to conduct two field efficacy studies. Both farms, “La Guaraní” and “Las Taperitas”, were located in Goya, Corrientes province in the northeastern part of Argentina where a low efficacy (based on sensitivity analysis) with ivermectin, fluazuron, fipronil and amitraz, against *Rhipichephalus microplus* in cattle, had been reported.

A total of 16 pregnant Braford cows (with young suckling calves) with an average body weight of 500 kilograms and with high tick infestations were selected for the first study in “Las Taperitas” on July 13^{th,} 2023. All animals were identified electronically for further traceability. The animals were randomly divided into two treatment groups of eight animals each. One group served as the fluralaner-treatment group while the other group served as untreated control group. The second study was conducted on the same day but in “La Guaraní”, following the same design with exception of number of animals, which were 18 Braford cows with an average body weight of 460 kilograms. The animals were randomly divided into two treatment groups of nine animals each. On Day 0, for all animals belonging to fluralaner-treatment group in both studies were treated with a single dose of EXZOLT 5% Pour-On by a veterinarian. The animals were treated at a dose ratel of 1mL/20 kg body weight as a pour-on application on the dorsal line of the animal as indicated on the label. The untreated control animals did not receive any treatment. The animals of the treatment groups and the control groups were kept separate, avoiding contact between them and possible licking. Animal welfare was ensured by avoiding unnecessary stress. In both studies, tick counts were conducted on individual animals (both sides of animals) on Days 5 and 14 after treatment.

Results

In both studies, onset of 100% efficacy, as demonstrated by absence of any viable tick, was observed starting from Day 5 post-treatment. On Day 14 after treatment in “La Guaraní”, all animals were still completely free from ticks. The injuries to the hides and hair from these animals, caused by ticks, were completely healed. On the same day in “Las Taperitas” no live ticks could be seen on the animals and only dead and dry debris was noticed on the areas where ticks were feeding.

Conclusions

It can be concluded that EXZOLT® 5% Pour-On is highly efficacious in controlling strains of *R. microplus* ticks resistant to commonly used ectoparasiticides in cattle in Argentina.

1182 - Dose confirmation of a novel fixed dose combination injectable (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) against naturally acquired gastrointestinal nematodes in US cattle

Author: Andrew DeRosa, Jase Ball , Angela Nadrasik, Jezaniah Tena, Frederico Moreira

Objectives
Macrocyclic lactone (ML) resistance in cattle gastrointestinal nematodes (GIN) is an increasing problem. Concurrent combination anthelmintic therapy incorporating an existing ML with a second drug class has been proposed to control cattle GIN while slowing the development of ML resistance.
Material and methods
Two dose confirmation studies were conducted to investigate the efficacy of a new fixed-dose combination injectable (FDCI) anthelmintic against common cattle GINs known to negatively impact production. The FDCI is formulated with 5 mg/ml doramectin and 150 mg/ml levamisole hydrochloride (HCl). Cattle enrolled in the two studies were sourced from either the Southern (Study 1, n = 30) or Midwest (Study 2, n = 36) United States. Animals with GIN infections confirmed by fecal egg count (FEC) were randomly allocated to one of three treatment groups. On Day 0, cattle with positive FECs on Day -5 (± 2) were weighed and administered a single subcutaneous injection of either saline (0.9% sodium chloride) at 0.04 ml/kg, 10 mg/ml doramectin at 0.02 ml/kg (to provide 0.2 mg/kg doramectin) or the FDCI at 0.04 ml/kg (to provide 0.2 mg/kg doramectin and 6.0 mg/kg levamisole HCl). On Day 14, fecal samples were collected, animals were euthanized, and worms were collected from the intestinal tract of each animal. Treatment efficacy was calculated using worm burdens and the fecal egg count reduction test (FECRT).
Results
Pre-treatment (Day -5, Study 1; Day -3, Study 2) mean FECs were 999.4 to 1136.2 eggs per gram (EPG) in Study 1 and 137.1 to 226.6 EPG in Study 2. The FDCI was active against cattle GIN populations in both studies, with FECRT ≥ 99.98% in both studies. Compared to saline-treated cattle, FDCI-treated cattle had significantly fewer adult and immature worms of all identified species on Day 14. In Study 1, Day 14 efficacy of the FDCI was 96.9 % for *Cooperia* spp. (*C. oncophora* (99.7 %) and *C. punctata* (95.9 %)), 99.1 % for *Nematodirus helvetianus,* and 99.8 % for *Ostertagia* spp.. In Study 2, the FDCI provided 100% efficacy against all adult GIN species identified, including all GINs identified in Study 1 and *Trichostrongylus axei.* The FDCI also provided 95.5 % efficacy against immature *Ostertagia* spp. and 100 % efficacy against immature *Cooperia* spp. (Study 2). Doramectin was effective against all adult cattle GINs (except *N. helvetianus*) in Study 2 but was only effective against adult *Ostertagia* spp. in Study 1. Additionally, doramectin was only effective against immature *Cooperia* spp. (and not immature *Ostertagia* spp.) in Study 2.
Conclusions
A single administration of the doramectin + levamisole HCl FDCI provides a new and effective approach to the treatment and control of common cattle GINs, including those exhibiting decreased susceptibility to doramectin alone.

1183 - Efficacy in US cattle of a novel fixed-dose combination injectable (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) against naturally acquired gastrointestinal nematode infections

Author: Andrew DeRosa, Jase Ball , Angela Nadrasik, Jezaniah Tena, Frederico Moreira

Objectives
Reports of macrocyclic lactone (ML) loss of efficacy suggest ML resistance in gastrointestinal nematodes (GINs) is a growing problem in the US cattle industry. Empirical and modeling data support combining an ML and second anthelmintic from a different drug class to help ML resistance development while effectively treating existing resistant parasite populations.
Material and methods
Here, we present a novel fixed-dose combination injectable (FDCI) solution for cattle that delivers 0.2 mg of doramectin and 6.0 mg of levamisole hydrochloride (HCl) per kg of body weight. Field studies were conducted at six sites across the Midwest United States to investigate the efficacy of a single administration of the FDCI in treating common cattle GINs. Cattle (n = 425) with GIN infections confirmed by fecal egg count (FEC) on Day -10 (±2) were randomly allocated to the control (saline) or treatment (FDCI) group. On Day 0, pre-treatment fecal samples were collected, and cattle were administered a single subcutaneous injection of saline (n = 106) or FDCI (n = 319). Post-treatment fecal samples were collected on Day 14. Fecal egg count reduction tests (FECRTs) were conducted using Day 0 and Day 14 FECs. Efficacy was evaluated using Day 14 FECs (FDCI-treated versus saline-treated). Within treatment, samples collected on Days 0 and 14 with ≥ 20 eggs per gram (EPG) were cultured for nematode larvae recovery and identification.
Results
Day -10 FECs for enrolled animals were similar between treatment groups. Coprocultures from cattle with FEC ≥ 20 EPG (n = 68 saline; n = 211, FDCI) on Day 0 showed the presence of *Cooperia punctata*, *Ostertagia* spp., *Haemonchus* spp., *C. oncophora*, *Oesophagostomum* spp. and *Trichostrongylus* spp. Day 14 FECs for FDCI-treated cattle were significantly reduced (0 to 3 EPG) compared to saline-treated cattle (0 to 1,252 EPG; $p \leq 0.0042$). At all study sites, the efficacy of the new FDCI was ≥ 99.4% and the FECR was 0.99 or 1.00. Day 14 coprocultures from control cattle showed infections of common GIN genera, confirming the efficacy of the FDCI against GINs in the field.
Conclusions
A single administration of the doramectin + levamisole HCl combination injectable effectively treats common and economically important cattle GINs.

1186 - Pharmacokinetic profiles of eprinomectin after a single subcutaneous or intramuscular injection in non lactating ewes

Author: Damien Achard , François Reynier, Hamadi Karembe, Nathalie Varinot, Reynald Magnier, Anne Geneteau, Pedro Pinho

Objectives

Eprinomectin is highly important to the dairy sheep industry as it can address internal and external parasitic infections with limited distribution in milk (zero-day milk withdrawal period). Recently, an injectable formulation has been licensed for subcutaneous administration in sheep ("Eprecis® 20 mg/mL Solution for Injection, Ceva Santé Animale"). Whenever possible, the injectable formulation should be preferred to the pour-on to limit the risk of under-exposure and environmental toxicity (Rostang *et al.,* 2022). Although subcutaneous route is the preferred way for many users, certain circumstances make intramuscular route more appealing. To gain more insights about the safety and efficacy of the intramuscular route, a comparative pharmacokinetic study was performed in non-lactating ewes.

Material and methods

Following a two-week acclimatation period, 24 non-lactating dairy ewes in good health were weighed one day prior treatment and randomized accordingly to be allocated into 2-treatment parallel groups (subcutaneous or intramuscular). At day 0, all animals received 0.2mg/kg of "Eprecis® 20 mg/ml" according to their group. Local reactions were assessed once daily for 1 week then every 2 days until disappearance of signs (corresponding to 2 consecutive observations with no signs). The following criteria were used (0 to 3 scoring): pain at injection, swelling, erythema, induration. Blood samples were collected at the following time points: D-1, 6 h, 8 h, 10 h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 96 h, and on D7, D14, D21, D28 and D35 after treatment. Plasma specimen analyses for eprinomectin were performed according to a specific LC-MS/MS method with a lower limit of quantification of 0.50 ng/mL. The pharmacokinetic analysis was performed using Phoenix® WinNonlin® 8.3 software (Certara, USA).

Results

Regarding the local tolerance, some signs were observed in 8 out of 24 animals: 5 animals after subcutaneous injection and 3 animals after the intramuscular injection. These signs were mostly swelling at the injection site during the first 3 days post-dosing. Following intramuscular administration of eprinomectin, the median time above 2 ng/mL was 7 days and the mean C_{max} was increased by 144% compared to the subcutaneous way route of administration. The $AUC_{0\text{-}7d}$ and $AUC_{0\text{-}t}$ were also increased by +71% and +26% after dosing intramuscularly compared to subcutaneously. The same inter-individual variability was reported for the pharmacokinetic parameters whatever the route of administration.

Conclusions

"Eprecis® 20 mg/ml" was well tolerated, whatever the route of administration. The pharmacokinetic profile of eprinomectin was different after intramuscular administration compared to the subcutaneous route as the rate and extent of absorption were increased by 144% for C_{max} and by 71% for $AUC_{0\text{-}7d}$. Taken together these results suggest that intramuscular route at the currently registered dose has a

good local tolerance and a good predicted efficacy in sheep. General tolerance and tissue residues should be further evaluated.

References

Rostang, A., Chartier, C., Jacquiet, P. (2022). L'usage de l'éprinomectine chez les petits ruminants. Le Point Vétérinaire. 434. 74-76.

1193 - Efficacy report of a fluralaner pour-on product against common ectoparasites infesting cattle in Brazil

Author: Daniel Rodriques, Tom Strydom , AJ Costa, JRS Martins, Fernado Borges, Luis Vettorato, Francisco Barufi, Heitor Amaral, Luara Abujarma, WDZ Lopes

Objectives

This report comprises the findings from efficacy studies of a novel pharmaceutical molecule, fluralaner (belonging to a new chemical class called the Isoxazolines), against important ectoparasites infesting cattle in Brazil.

Material and methods

In total, thirteen studies were conducted with a 5% fluralaner pour-on formulation (Exzolt® 5%). Out of thirteen studies, six studies investigated the effectiveness of Exzolt® 5% against *Rhipicephalus microplus* (field and controlled studies), four against *Cochliomyia hominivorax* larvae, one against *Dermatobia hominis* larvae and two against *Haematobia irritans* flies. Prior to treatment in each study, appropriate pre-treatment parasite counts were done, and the selected animals were assigned to one of two treatment groups in accordance with a randomized block design. A negative control Group (T02) was included in all studies, in which animals were treated with a placebo formulation. The animals of Group T01, in each study, were treated with Exzolt® 5% at a dose of 2.5 mg fluralaner/kg bodyweight (BW). In all studies, animals belonging to the two treatment groups remained separated to avoid the possibility of cross-contamination between the placebo and fluralaner treated animals.

Results

Efficacy against *R. microplus* was assessed in experimentally infested animals, where the therapeutic efficacy for Exzolt® 5% was found to be above 98% at four days post treatment (DPT). A persistent efficacy (>90% efficacy) against repeated infestations with *R. microplus* was observed for up to 79 DPT. Efficacy of the product was also assessed in five field studies conducted in animals naturally infested with *R. microplus*. In these studies, a ≥98% therapeutic efficacy was demonstrated at all study sites by 7 DPT and a persistent efficacy (>90% efficacy) was observed for 42 DPT at 3 sites, 49 DPT at 1 site and 56 DPT at another site. Efficacy of Exzolt® 5% was also assessed against *C. hominivorax*-induced myiasis in four study sites, to assess both the preventative efficacy against myiasis (two sites) and the curative efficacy in established myiasis (two other sites). Results showed that the product prevented *C. hominivorax* eggs developing to the larval stage thus mitigating the development of myiasis in artificially created wounds for 7 and 14 DPT. No active *C. hominivorax* larvae were observed by 3 DPT in animals with pre-existing myiasis. Additionally, efficacy of Exzolt 5% was assessed against *D hominis*. The therapeutic efficacy of the product against *D. hominis* larvae was 98% at 3 DPT, while the persistent efficacy (>90% effectiveness) lasted for up to 70 DPT. Finally, two field efficacy studies were conducted to evaluate the efficacy of Exzolt® 5% against *Haematobia irritans* under field conditions. In these studies, following a single administration of the product, a therapeutic efficacy of ≥90% was observed within the first day of treatment at 2 study sites, while a persistent efficacy (≥90%) was observed for 7 DPT at one site and for 21 DPT at another site.

Conclusions

Overall results from these studies confirm that Exzolt® 5% provides a therapeutic efficacy against the most important ectoparasites infesting cattle in Brazil, along with an impressive persistent efficacy against re-infestation. The novel active pharmaceutical ingredient, fluralaner, provides a new treatment option for farmers to control these cattle ectoparasites, especially where there is resistance to other chemical classes. An effective control of ectoparasites, in turn, will improve overall cattle health, well-being and production.

1194 - Different strategies for using fluralaner pour-on product (Exzolt 5%) to control Rhipicephalus microplus on taurine cattle breeds in a tropical region of Brazil

Author: Daniel Rodriques, Tom Strydom , LM de Aquino, DMB Zapa, Siddartha Torres-Rico, L L Ferreira, Francisco Barufi, Heitor Amaral, Fernado Borges, Tiago Gallina, R P Mendonca, V E Soares, C M O Monteiro, WDZ Lopes

Objectives
This study aimed to evaluate the effect of two different treatment protocols with a newly approved commercial pour-on formulation containing fluralaner (Exzolt 5%) for the strategic control of *Rhipicephalus microplus* infestations in cattle and on pastures in a tropical climate region of Brazil where up to five generations of this tick species can occur per year.
Material and methods
Forty-five cattle, naturally infested with *R. microplus* ticks were selected for the study and divided into three experimental treatment groups of 15 cattle each. Group T01 was treated with Exzolt 5% at a dose rate of 2.5 mg/kg body weight every 42 days. Group T02 was treated with Exzolt 5% at the same dose on Day 0, followed by weekly re-treatment based on visual presence of tick infestations. Group T03 (untreated control group) was left untreated but received palliative treatment with a commercial acaricidal spray formulation when the group mean tick count was ≥30. Weekly counts of adult female *R. microplus* ticks (4.5 to 8 mm in size) were performed on animals until day 343 after first treatment, and larval counts on pastures were also performed on Days 0, 30, and 60 and every 30 days until Day 330 post first treatment.
Results
Group T01 received 6 treatments with the fluralaner pour-on formulation (every 42 days) while Group T02 was only treated 4 times over the study period. In the control group (T03), it was necessary to perform eight palliative acaricide treatments with the spray formulation. The animals on Groups T01 and T02 showed lower mean tick counts ($p \leq 0.05$) than the control group (T03) on 28 and 27 of the 49 tick count days respectively. In the paddock (19.5ha) where the animals treated with Exzolt 5% were kept, the number of tick larvae decreased from 32 to 5 during the study. In the paddock (19.5 ha) where the animals were kept as controls, *R. microplus* larvae counts increased from 41 to 1458 larvae.
Conclusions
The study showed that depending on the treatment criteria (fixed interval of 42 days or as needed based on the visual presence of *R. microplus* ticks) adopted for the re-treatment of tick infested cattle with Exzolt 5%, the number of product administrations per year could be lower to control *R. microplus* ticks than traditional treatment protocols. The reduced frequency of re-treatment would translate into not only a reduction in the average cost of acaricide treatments per year but will also reduce the exposure of *R. microplus* populations to the active ingredient resulting in slowing-down resistance development.

1195 - Evaluation of the effect of Fenbendazole and Toltrazuril administration at birth or at 45 days of age on weight gain and incidence of diarrhea in beef calves in Brazil

Author: Daniel Rodriques, Tom Strydom , M de O Marques, Joao Garcia, Lais Vieria, Henderson Ayres

Objectives

The study was conducted to measure weight gain, adjusted weight and incidence of diarrhea from birth to weaning in suckling beef calves after administration of Fenbendazole and Toltrazuril (Panacoxx®) on the day of birth or at 45 days of age.

Material and methods

From the day of birth (Day 0), after identification by ear tag, navel healing, weighing and administration of 1ml of Doramectin s.c (200mcg/kg), a total of 586 calves were divided into 4 experimental groups, in a 2x2 factorial design: T1: Control group without treatment, T2: treatment on the day of birth, T3: treatment on the 45th day of age and T4: treatment on the day of birth and on the 45th day of age. Animals of Groups T2, T3 and T4 were concomitantly treated with a product containing Fenbendazole (2,5%) and Toltrazuril (7,5%) (Panacoxx® 1ml/5kg). About 50% of the animals in each group had fecal samples collected for oocyst counts eggs per gram of feces (EPG) and oocyst per gram of feces (OOPG) evaluation at the following times: Day 45-50 (in pre-fixed-time artificial insemination (FTAI) management), Day 60 or 68 during (FTAI) protocol and Day 150-160.

Results

There was no difference between the average daily weight gain (ADG) and adjusted weights between different experimental groups. The average daily weight gain (ADG) at 240 days for T1 was 0,84kg (± 0,01), for T2 0,83kg (± 0,01), for T3 0,84kg (± 0,01) and for T4 0,83kg (± 0,01) (P value 0,95). Treatment of calves on the day of birth (T2) and at 45 days (T3) with Panacoxx® showed a lower occurrence of diarrhea compared to the control groups.

Conclusions

The use of Panacoxx on the day of birth (T2) was more efficient in reducing the incidence of diarrhea associated with Eimeriosis, when compared to treatment of calves on day 45 days of age (T3).

1197 - Coccidiosis control recommendations by Veterinarians in the Animal Health Ireland parasite control programme

Author: Colin Grimes

Objectives
To examine Targeted Advisory Service on Animal Health (TASAH) recommendations on management of coccidiosis
Material and methods
Between April and December 2022, 14,879 recommendations on management of parasites in cattle were recorded. Of these, 710 related to coccidiosis. Recommendations on coccidiosis were broadly categorised into Diagnosis (monitoring for clinical signs, faecal sampling), Non-Pharmaceutical Management (hygiene changes) and Pharmaceutical Management (anticoccidial drugs).
Results
Advise from veterinarians often contained more than one piece of advice, the most common advice in descending order were: pharmaceutical management (recommended 450 times, 63%), diagnosis (269 times 38%) then non-pharmaceutical management (224 times, 31%). The most common time advised by veterinarians for the use of anticoccidials of coccidia was either three weeks after birth or arrival of the animals onto the farm. The most common diagnostic advice give was the use of faecal sampling which was advised 197 times.). Rotation of pasture or movement of water/feed troughs were the most commonly advised non-pharmaceutical management recommendations (42 times).
Conclusions
The recommendations given largely conform with the international accepted advice regarding the treatment and control of coccidiosis i.e., targeted use of anticoccidials based on recent clinical history on the farm, clinical signs and faecal sampling and the use of management changes including appropriate cleaning and disinfection, paddock rotation and the avoidance of poached ground.

1205 - Clinical Monitoring of Cattle Inoculated with Virulent or Attenuated Babesia bigemina Strains

Author: Tomás V. Santamaria, Julio V. Figueroa, Rebeca M. Santamaria, Grecia Martínez, José J. Lira, J. Antonio Álvarez, Carmen Rojas, Juan J. Ojeda

Objectives

Bovine babesiosis, caused by the protozoan *Babesia bigemina*, is one of the most important hemoparasitic diseases of cattle in Mexico and the world. An attenuated *B. bigemina* strain maintained under in vitro culture conditions has been used as a live attenuated vaccine; however, the biological mechanisms involved in attenuation are unknown. The objective of this work was to carry out comparative clinical and serological monitoring of animals inoculated with virulent or attenuated strains of *B. bigemina*.

Material and methods

With the approval by the Institutional Subcommittee for the Care and Use of Experimental Animals (SICUAE.DC-2022/1-3) of the College of Veterinary Medicine at UNAM, Mexico, seven cattle were used, one of which was splenectomized to reactivate the virulent strain previously cryopreserved in liquid nitrogen. The remaining 6 cattle were randomly grouped into Groups I (GI), inoculated with $1x10^8$ erythrocytes infected with *B. bigemina* virulent strain, and GII inoculated with $1x10^8$ erythrocytes infected with *B. bigemina*, attenuated strain, derived from in vitro culture. Daily monitoring included the recording of rectal temperature (°C) value and collecting blood to determine Packed Cell Volume (PCV) and Percent of Parasitized Erythrocytes (PPE). In addition, the Indirect Fluorescent Antibody Test (IFAT) and an iELISA were used.

Results

A PPE >9% was determined in the splenectomized calf, obtaining biological material for GI inoculation. The IG animals showed severe clinical signs, associated with acute babesiosis, with fever >41°C, haemoglobinuria, PPE >5% on day 5-6 Post-Inoculation (PI) and decrease in PCV from day 4 PI, with a minimum PCV value of 11.3% at the end of the monitoring. Animals were treated with babesiacide to prevent death. The GII cattle did not present fever or signs of acute babesiosis, presenting only a slight decrease in PCV (HT value of 25%), and PPE <0.1%. No GII cattle required treatment. Seroconversion was identified on day 7 PI, with a maximum antibody titter of 1:1280 on day 14 PI in the GI. In the GII, a maximum antibody titter of 1:2560 was determined.

Conclusions

It is concluded that the strain kept in cryopreservation maintains its virulence when reactivated in a splenectomised calf, observing severe clinical signs in the inoculated animals. Whereas, the attenuated strain, maintained in in vitro culture and used as a live vaccine, has not reverted to virulence, is safe and immunogenic.

1239 - Comparative efficacy of three different doses of paromomycin administered orally to treat cryptosporidiosis in experimentally infected dairy calves

Author: Damien Achard , Naomi Isaka, Marie Cron, Adélie Goujas, Thomas Blondel, Jean-François Collin, Jiří Smola, Břetislav Koudela

Objectives

Cryptosporidiosis is a major parasitic disease of newborn calves affecting their health, growth and welfare and responsible for significant economic losses for cattle famers. Current treatments for sick calves are scarce. Paromomycin is a valuable oral treatment option (Brainard *et al.,* 2020, 2021) although data has been limited to fully support its use for treatment. In addition, the literature offers little guidance about the dose for which a clear benefit is observed in affected calves. To address this issue, the effects of three different dose of paromomycin administered as an oral solution were explored in dairy calves experimentally infected with cryptosporidia.

Material and methods

A GCP study involving 35 healthy Holstein neonatal calves was performed according to a monocentric, blinded, randomised design, with 4 parallel groups comparing three different oral doses of paromomycin (Gabbrovet Multi®, Ceva Santé Animale) to a negative control group. Calves were aged 1-2 days old at arrival, 36 to 54 kg and had fair passive immunity status (serum total IgG > 10 g/L). After 24h of acclimatization, each calf was experimentally infected orally once with a dose of $1x10^6$ sporulated oocysts of *Cryptosporidium parvum* (DC_0). Calves were included and treated according to their group if they were 3-10 days old after challenge and had faecal score ≥ 1 and positive oocyst count on the same study day (DT_0). Treatment groups were as follows:

- 9 calves were left untreated (control)
- 9 calves were treated with Gabbrovet Multi® at 75 mg/kg BW/day for 5 days (group 75)
- 8 calves were treated with Gabbrovet Multi® at 100 mg/kg BW/day for 5 days (group 100)
- 9 calves were treated with Gabbrovet Multi® at 150 mg/kg BW/day for 5 days (group 150)

The following parameters were monitored at fixed times during the 21 days of the study: fecal score (0-2), general health observation (0-3), hydration score (0-3), number of oocysts per gram of dry faeces, bodyweight and mean daily body weight gain (MDBWG). Percentages of calves cured at DT_5, diarrhoea observations (DT_0 - DT_{21}) and MDBWG (DC_0 - DT_{21}) were used to compare the efficacy between treatment groups with cure defined as calves with all clinical scores = 0, and diarrhoea regrouping calves with fecal score of ≥1. Evolution of the mean oocyst count in each group was assessed daily from DT_0 to DT_5. The statistical unit was the calf and the 5% level of significance ($p < 0.05$ for two-sided tests) was used to assess statistical differences.

Results

At DT_5, no calf was cured in the control group; a dose effect was observed in treated groups with 44.4%, 62.5% and 88.9% of calves cured in groups 75, 100 and 150

respectively. Through the study, odds ratio (OR) for diarrhoea were found 5.67 times superior in the control group compared to calves in the group 150 [CI_{95}=2.63;12.24]. A significant effect on diarrhoea observations was also observed in calves in the group 100 in comparison with the control group although with a more limited impact (OR=2.32, [CI_{95}=1.08;4.97]). Calves from group 100 were found with OR for diarrhoea 2.45 times greater than calves in the group 150 [CI_{95}=1.11;5.40]. No effect was observed on diarrhoea observations in calves in the group 75 when compared to control group (OR=1.47; [CI_{95}=0.70;3.07]). MDBWG was statistically higher only in group 150 compared to the untreated group (0.67 *vs.* 0.53 kg/day; p=0.0192). In group 150, the mean oocyst count was always lower compared to all the other groups during the treatment's phase.

Conclusions

In this study, the highest dose of paromomycin (150 mg/kg per day for 5 days) was associated with high clinical cure 24h after completion of the treatment, an important decrease in diarrhoea observations and oocyst counts, and a positive impact on the mean daily body weight gain. These findings are in line with those described in a recent multicentric European field trial (Achard *et al.,* 2022). In contrast, a lower dose of paromomycin (75 mg/kg per day for 5 days) resulted in lower clinical cure, no effect on diarrhoea observations and no impact on MDBWG. These results suggest that the high dose of paromomycin (150 mg/kg) is the most efficient when treating neonatal calves sick from cryptosporidiosis.

1282 - Svanovir® kit application for identifying >>Ostertagia ostertagi<< exposure in southern mexican cattle.

Author: Josué Gómez Estrada, Cintli Martínez-Ortiz-de-Motellano , Nadia Florencia Ojeda Robertos, Enrique Reyes Novelo, Laura González Reyes, Roger Iván Rodríguez Vivas

Objectives
The study aimed to assess a commercial kit's efficacy in detecting *Ostertagia ostertagi* antibodies in milk from cows across three ecological zones in southern Mexico. Additionally, it sought to evaluate the feasibility of the kit's application.
Material and methods
Three ecological zones of southern Mexico were included, which are in Chiapas and Veracruz, covering the northern and southern zones. Five dairy farms were included in each of the zones. From a total of 300 dairy cattle, including Bos taurus and Bos indicus species, only 258 viable samples were obtained. During the first milking, milk samples were collected for antibody detection. One month later, the sampling was repeated using the same animals to contemplate the effect of dehiding the larvae in hypobiosis on the test result. Samples were taken directly from the udder in sterile 50ml conical tubes that were properly identified. Before sampling, the teats were washed and disinfected, then the first two streams were discarded, and 10 ml were collected from each quarter. They were kept refrigerated in a cooler and transported to the Integrated Health Management (IHM) Laboratory of Boehringer Ingelheim, located in Guadalajara, Jalisco. The SVANOVIR® *O. ostertagi*-Ab kit was used for antibody level analysis. The data were analyzed to obtain the prevalence and its confidence intervals through the Clopper-Pearson method by farm and by ecological zone. Subsequently, the association between ecological zones was analyzed using Fisher's test. All analyses were performed with the Quantitative Parasitology Web package.
Results
The total prevalence of infection with *O. ostertagi* in the cattle evaluated was 58.5% (CI 95% = 52.3–64.6). The northern Veracruz zone had a prevalence of 59.2% (58/98, CI 95% = 48.8–69). The southern Veracruz zone had 60.8% (73/120, CI 95% = 51.5–69.6). Chiapas had a relatively lower prevalence of 50% (20/40, CI 95% = 33.8–66.2). Overall, the prevalence among the ecological zones studied was statistically similar (P = 0.497).

Conclusions
Nemaodes of the species *O. ostertagi* cause bovine ostertagiosis, a disease in cattle. In Mexico, the prevalence has been estimated at between 8 and 13%, with losses of approximately 90 kg per cattle in the wet season. The disease represents a challenge in terms of accurate diagnosis since there are two types of manifestations. Type I, which occurs in the abomasum lumen, where male and female helminths feed, mate, and lay eggs to continue the cycle. Diagnosing and identifying *O. ostertagi* proves challenging due to the necessity for larval culture, extending the diagnosis to 12–15 days. Type II, involving prolonged hypobiosis of larvae stage (L4) in the abomasal mucosa. That complicates diagnosis during the dry season and/or winter.
This study revealed a cattle prevalence estimate ranging from 50 to 60%, diverging from prior Mexican studies reporting maximum values of 15%. Many studies on the

prevalence of gastrointestinal nematodes use diagnostic techniques such as larval culture, egg measurement, or identifying adult nematodes during necropsy. Evaluating *O. ostertagi* antibodies in milk is a promising alternative to larval culture. This approach simplifies the diagnostic process for parasite detection in dairy cattle, providing advantages in terms of time efficiency, practicality, and logistical convenience.

The prevalence of *O. ostertegi*-positive serum remained similar between the studied areas of Veracruz (~ 60%) and Chiapas (50%). The use of a commercial kit for the detection of antibodies in milk proved to be an effective diagnostic tool, being an alternative to larval culture, which contributes to more effective control strategies. In addition, ostertagiosis should be regarded as a disease of importance in the bovine area in these evaluated zones to implement adequate control strategies to minimize economic losses. The results obtained in this study promote the use of more effective practices in parasitic diagnosis in southern Mexico.

1291 - Relative expression of the Glutathione peroxidase gene of Haemonchus contortus infective larvae exposed to isoguaiacine and norisoguaiacine from Artemisia cina

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Objectives

To quantify the relative expression of the glutathione peroxidase gene (Hc29) in *Haemonchus contortus* infective larvae previously exposed to isoguaiacine and norisoguaiacine from *Artemisia cina.*

Material and methods

Leaves and stems of the pre-flowering Artemisia cina were obtained and macerated for *n*-hexanic extract by 48 hours, the *n*-hexane extract was concentrated under reduced pressure and subsequently stored in a desiccator before use.

The extract was separated by chromatographic techniques using silica gel (70 – 230 Mesh, Merck) in normal phase (Merck, USA), the mobile phase was n-hexane and ethyl acetate. The metabolites (isoguaiacine and norisoguaiacine) obtained are monitored and analyzed by thin layer chromatography and will be concentrated and dried in a desiccator to be used in in-vitro assays. The in vitro assay followed the next design: 4000 infective larvae of H. contortus was used per group so, Group 1: mixture of isoguaiacine and norisoguaiacine at 2mg/ml (n=4), group 2: mixture of isoguaiacine and norisoguaiacine at 4mg/ml (n=4), group 3: distillated water (n=4), group 4: hydrogen peroxide (n=4), group 5: ivermectine (5mg/ml) (n=4). The infective larvae were exposed for 24 h and after RNA was extracted.

Treated infective larvae will be recovered and centrifuged at 1000 g for 5 minutes, unsheathing with 0.185% sodium hypochlorite and crushed using liquid nitrogen and pistil, then there were suspended in 1 mL of Trizol (Thermo Fisher Scientific, USA) for 24 h. DNAc was elaborated. Oligonucleotides were designed for the GPX (*H. contortus* L3 glutathione peroxidase) and β-tubulin genes using the sequences reported by NCBI. The sequences were used for the RT-qPCR technique (Corbett Research Roto-Gene 6000) using thirty cycles of amplification with an initial incubation at 95 °C for 5 minutes, followed by denaturation at 95 °C for 15 seconds and alignment at 63 °C for 15 seconds. The quantification of relative expression gene was quantified by ΔΔCT method using the constituent gene as control group. The relative expression of the Glutathione peroxidase (GPX) gene values was reported with Student t proof ($p < 0,05$; Qiagen plataform, https:// www. qiagen com).

Results

The exposed infective larvae of H. contortus at isoguaiacine and norisoguaiacine from *A. cina* did not induce damage into the larvae. GPX gene was underregulation respect to constituent gene (β-tubulin).

Conclusions

Lignans as isoguaiacine and norisoguaiacine from Artemisia cina caused underregulation of GPX gene in infective larvae of Haemonchus contortus exposed.

1297 - Identifying when anticoccidial metaphylaxis should be applied on Spanish dairy and meat sheep farms.

Author: Ignacio Ferre Perez, Tom Strydom , Lourdes Brandariz-Amores, Jorge Gutiérrez, José Luis Blasco-Castello, Laura Elvira, Luis Miguel Ortega-Mora

Objectives

The aim of this study was to investigate the *Eimeria* spp. infection dynamics in lambs from representative dairy and meat Spanish farms to identify clinical and parasitological criteria useful to decide the optimal moment for metaphylactic treatment. Ovine coccidiosis is a worldwide parasitic disease of great veterinary importance due to its high economic impact. Lambs are infected by the ingestion of sporulated oocysts, experiencing diarrhea and low growth rates. The control of ovine coccidiosis has been based on the prophylactic use of oral drugs such as decoquinate and diclazuril. However, according to the new European legislation on veterinary drugs (Regulation (EU) 2019/6) the administration of authorized drugs for ovine coccidiosis can only be done metaphylactically once infection has occurred.

Material and methods

Three dairy and three meat sheep farms were included in the study. The inclusion criteria was: a) on-farm veterinarian (dairy) or weekly veterinarian visits (meat), b) representative management of the sector, and c) treatment with diclazuril (Vecoxan®) against coccidiosis. Information on management and risk factors for coccidiosis were collected through on-farm surveys. On each farm, seven sentinel lambs were selected for fecal sampling and collection of clinical data (body condition, fecal consistency and staining of the back end) at weaning, 10-15 days after weaning and five days after metaphylactic treatment, as well as 20 days before weaning on meat farms. Additionally, the samples could be collected at any moment if clinical signs compatible with coccidiosis appearance and did not coincide with the scheduled dates. *Eimeria* oocyst counts were performed using the modified McMaster technique and species identification was done in positive samples. The administration of diclazuril was based on the laboratory analysis and the clinical signs of the lambs.

Results

On dairy sheep farms, no *Eimeria* oocysts were detected at weaning and the lambs did not have clinical signs compatible with coccidiosis, most likely due to the strict hygienic conditions in nursing facilities, so the administration of diclazuril before weaning would not be justified. Infection was detected after weaning and there were differences in the clinical impact on each farm, mainly due to the differences in hygienic measures of facilities where lambs were kept after weaning. Diclazuril was administered and its efficacy was proven at five days after treatment by the reduction of oocyst output and clinical data improvement.

On meat sheep farms, samples were collected 15 days before weaning but only samples from a farm where lambs were reared outdoors showed fecal oocysts output. Higher oocyst counts were detected after weaning in lambs reared indoors and outdoors on meat farms than those observed on dairy farms. This is possibly due to the poorer hygienic conditions found on meat farms and the coexistence of adult sheep and lambs, which could play an important epidemiological role. Diclazuril was administered to lambs on all three meat farms after weaning, detecting afterwards very low oocyst

counts and an improvement of clinical signs. Pathogenic *Eimeria* species were detected on all meat and dairy farms, always with other non-pathogenic species.

Conclusions

Due to the new European legislation and drug resistance increase, procedures as those used in the present study should be introduced, since they are not expensive, simple to carry out and give satisfactory results. In conclusion, monitoring parasitological and clinical parameters in a group of sentinels could help determine the optimal time to apply metaphylactic treatment using diclazuril, as well as obtain relevant information to modify cleaning and disinfection strategies and lamb management.

1298 - How coccidiosis is managed on Spanish sheep farms: a questionnaire-based survey

Author: Luis Miguel Ortega-Mora, Tom Strydom , Roberto Sánchez-Sánchez, Jorge Gutiérrez, José Luis Blasco-Castello, María Marcos-Santamaría, Santiago Cano-Alsua, Laura Elvira, Ignacio Ferre Perez

Objectives
To investigate the common control measures conducted for coccidiosis in Spanish sheep farms. Ovine coccidiosis is a worldwide parasitic disease caused by *Eimeria* spp. associated with high economic losses in lambs showing diarrhea and low growth rates. Control measures should be directed to reduce infection pressure and stress on the animals and appropriate diagnosis and strategic treatment.
Material and methods
A questionnaire-based survey was carried out in 2022 where 154 veterinarians and 173 farmers were included in a non-random convenience sampling study.
Results
Coccidiosis was considered as a relevant disease by 34% of veterinarians and farmers. The period of greatest risk was different between production systems. In meat flocks and feedlots it was mainly early after weaning (7-15 days after weaning) but later in dairy flocks (1-2 months after weaning). The absence of cleaning and disinfection measures was identified as a risk factor by 51% of the veterinarians. In addition, the higher incidence of coccidiosis in flocks with large number of animals and the overcrowding effect were highlighted by 22% of respondents. The use of fecal oocyst counts for diagnosis was unusual in 70% and 84% of the veterinarians and farmers, respectively. Dairy flocks usually housed a larger number of animals under intensive conditions and implemented more frequently control measures for coccidiosis than meat flocks.
Anticoccidial drugs were used in 79% of the flocks and mainly based on clinical criteria (74-82% of them). Oral mono-dose anticoccidials (diclazuril and toltrazuril) were more commonly used than medicated feed (decoquinate). Drug administration was mainly associated with clinical signs appearance. Differences between meat and dairy flocks were identified related to management. In dairy flocks there was a better implementation of management measures (cleaning and management measures to reduce stress in the animals), which could be the reason for the emergence of coccidiosis long after weaning.
Conclusions
These results show the need for anticoccidial treatments improvement according to the new regulatory framework in the EU. The rational use of anticoccidials may help to mitigate the impact of coccidiosis in Spanish sheep flocks.

1307 - Preventative/Prophylactic activity of isometamidium against Trypanosoma vivax in experimentally infected cattle in Brazil.

Author: Daniel Rodriques, Tom Strydom , DMB Zapa, L M Heller, Lidia Mendes de Aquino, WDZ Lopes, Luis Vettorato, Heitor Amaral, Francisco Barufi

Objectives

This study aimed to determine the preventative/prophylactic activity of a formulation containing isometamidium (Trypamizol® - MSD Saúde Animal) in cattle treated at a dose rate of 1 mg/kg body weight, against an experimentally induced *Trypanosoma* (*T.*) *vivax* infection.

Material and methods

Eighteen healthy cattle, negative for the presence of *T. vivax*, using the Woo, Brener and cPCR techniques, were selected at D-130. Animals were divided into three groups of six animals each, based on the body weight measured on Day -121. In each group, treatment with an experimental formulation of isometamidium (Trypamizol® - MSD Saúde Animal) was carried out as follows: Groups T01 and T02 were treated respectively on Days -90 and D-60. All treatments were performed by deep intramuscular injection at a dose of 1mg/kg body weight. A control group of 6 animals (Group T03) was included in the study and treated with a saline solution in a way that two different animals from this group were treated on each of the days when treatment was administered to groups T01 and T02. On day 0 of the study, all animals were infected intravenously with a suspension containing $1x10^6$ trypomastigotes *of T. vivax*.

Results

The results demonstrated that isometamidium (Trypamizol® - MSD Saúde Animal), administered intramuscularly at a dose rate of 1 mg/kg to the calves, was effective as a prophylactic treatment against *T. vivax*. The combination of diagnostic techniques (blood smears, Woo, Brener and cPCR) made it possible to diagnose the presence of *T. vivax* in all calves in the control group. No parasites could be detected in calves from Group T01 and T02 treated with isometamidium at -90 days and -60 days by any of the diagnostic techniques used (smear, Woo, Brener and PCR).

Conclusions

It can be concluded that the product Trypamizol®, administered intramuscularly to cattle at a dose of 1 mg isometamidium/kg body weight, showed 100% prophylactic activity for 90 days against *Trypanosoma vivax* in experimentally infected cattle. In this way, carrying out treatment in D-90 before contact with the parasite makes it possible for animals not to develop the disease.

1313 - Efficacy of a subcutaneous injection of eprinomectin in lactating goats experimentally challenged with gastrointestinal and lungworms

Author: Damien Achard , Pedro Pinho, Sarah Thomson, David Reddick, Rick Brunner, Dana Campbell, Alexandra Beck, Naomi Isaka, Anne Trotel, Hamadi Karembe

Objectives

Health and productivity of dairy goats continue to be impacted by gastrointestinal nematodes (GIN) and lungworms (LW). Eprinomectin (EPN) is frequently selected for treatment as it is considered effective and without milk withdrawal period. However, some factors such as the lactation status can have an impact on EPN pharmacokinetics and potentially its efficacy. For instance, reduced bioavailability has been reported for topical EPN in lactating goats when compared to dry goats (Dupuy *et al.,* 2001). To evaluate if this can alter the efficacy of an injectable solution of eprinomectin (Eprecis® 2%, Ceva Santé Animale), a study was performed in lactating goats using the dose currently registered in cattle, sheep, and goat (0.2 mg/kg).

Material and methods

This study was a blinded, randomized, controlled trial performed according to VICH guidelines (GL7 and GL14). The protocol was reviewed by the Moredun Animal Welfare and Ethical Review Board, and the study run under the UK Home Office Project Licence (No PFA7E7AD6) following Animal Scientific Procedures Act 1986. Eighteen (18) worm free lactating goats from a conventional dairy goat farm were included and experimentally challenged on Day-28 with a mixed culture of infective gastrointestinal and lung nematode larvae (*Haemonchus contortus, Trichostrongylus colubriformis, Teladorsagia circumcincta, Dictyocaulus filaria*). Average numbers of parasites inoculated complied with the recommended numbers for parasite infection models for anthelminthic evaluation in goats. The isolates used were recent European strains isolated from field (except *T. colubriformis* from laboratory isolate). At D-1, faecal samples were collected to confirm patent infestation in all animals (minimum > 150 EPG). Fifteen animals were ultimately enrolled in the study, as three goats shown a faecal egg count below the shedding threshold. On D0, goats were randomly allocated based on their D-1 faecal egg count (FEC) and on D0 body weight into two groups of nine goats; group 1 was treated with Eprecis® 2% at 0.2 mg/kg BW by subcutaneous injection while group 2 remained untreated. The goats were milked once daily, and their general health was assessed twice daily from arrival until the end of study. Any sign of abnormal behaviour or a change in feeding habits was reported. Faecal egg shedding was regularly monitored from D0 to D14, with a total of six faecal egg counts, performed at D3, 5, 7, 9, 11 and 14. On D14, all goats were euthanised as required by VICH guidelines, and the abomasum, small intestine and lungs removed, processed and sub sampled, to record the number and species of worms. To assess the effect of Eprecis® 2%, the nematode counts at necropsy of treated animals were compared to those of control animals, using Wilcoxon Tests, with significance set at the 0.05 level. The effectiveness of Eprecis® 2% against each worm species was calculated as the difference in geometric means of the counts between the control group and the treatment group at D14, expressed as a percentage based on the geometric mean of the control group counts. Statistical analyses were performed using SAS version 9.4 software.

Results

The treatment was well tolerated. After treatment, mean FEC decreased in the treated group and remained lower than 3 EPG until the end of the study while mean FEC in the control group remained higher than 849 EPG. At D14, goats in the treated group had very limited or zero total worm counts whereas all animals from the control group had high worm burden. Measured efficacy was 100.0% against *H. contortus* and *T. colubriformis,* 99.9% against *T. circumcincta* and 96.8% against *D. filaria*. The mean body weights in both groups increased slightly from D0 to D14 (respectively of +2.8 kg in the treated group and +1.6 kg in the control group).

Conclusions

Eprinomectin injection (Eprecis® 2%), administered at the label dose (0.2 mg/kg) is highly effective and safe against gastrointestinal and lungworms in lactating goats. These findings are in line with the results obtained after experimental GIN infection and treatment at label dose with Eprecis® 2% in dry goats (Brique-Pellet *et al.*, 2017) and thus suggest that lactational status of goats does not impact the efficacy of this injectable eprinomectin solution.

1353 - Transcriptomic profile of peripheral white blood cells in cattle inoculated with virulent or attenuated strains of Babesia bigemina

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Objectives

Bovine babesiosis is caused by intraerythrocytic parasites of the *Babesia* genus. *Babesia bigemina* is the most widely distributed species in the world, with a global prevalence of 29%. The high prevalence and morbidity imposed by the pathogen in cattle place the disease as an important constraint to monitor in developing countries. In the present study, differentially expressed genes were identified in peripheral white blood cells derived from cattle inoculated with a virulent or an attenuated strain of *Babesia bigemina*, the latter used as a live immunogen that has been shown to confer a protective efficacy ≥80%. A deeper understanding of the host's immune response to infection with a virulent or an attenuated strain could help in the development of more effective approaches for the control of bovine babesiosis. The objective of this study was to compare the expression profile of genes related to immune response present in buffy coat layer cells derived from peripheral blood of bovines inoculated with a virulent or an attenuated *Babesia bigemina* strain.

Material and methods

Two study groups were randomly formed, each consisting of three *Bos taurus* steers, > 12 months old, free of infectious diseases such as brucellosis and tuberculosis, from a geographical region free of the *R. microplus* tick vector, and with negative results for *B. bigemina* and *B. bovis* by using indirect fluorescent antibody tests and nested endpoint PCR assays for both species. Group I (GI) cattle were inoculated with a virulent strain of *B. bigemina;* Group II (GII) were inoculated with an in vitro culture-derived attenuated *B. bigemina* strain. At peak parasitemia, as determined by microscopic analysis of Giemsa-stained blood smears, blood was aseptically collected and the upper buffy coat layers present in the packed cells were aspirated after centrifugation in a clinical refrigerated centrifuge. After RNA extraction, library construction was carried out using the commercial TrueSeq® RNA Library Preparation kit, while the Illumina® NextSeq 500 System platform was used for sequencing. The cDNA libraries created for each sample were sequenced in 2x75 cycles with an approximate total of 10 million readings per sample. Transcriptomic analysis (RNA-seq) was performed, followed by bioinformatics analysis and Gene Ontology (GO)-based term enrichment.

Results

High-throughput sequencing consisting of 13,400,000 – 15,750,000 and 9,504,000 – 9,656,000 sequence reads were obtained from the buffy coats of GI and GII cattle, respectively. For the differential expression analysis, only sequence reads that mapped against the reference *Bos taurus* genome (GCF_002263795.1) were used. Preliminary analysis of the information showed that at least 371 differentially expressed genes (DEG) were identified between experimental GI and GII cattle. This, as a result of the integration with bioinformatic anylises performed in platforms DESeq2, edgeR and NOIseq. Additionally, 526 genes were detected only by NOIseq, 100 by edgeR and 13 by DESeq2, with the largest number of concordant genes (420) between edgeR and NOIseq. The preliminary results of this research indicate that the in vitro culture-derived

attenuated *B. bigemina* strain induces global transcriptomic changes in inoculated susceptible cattle that are related to at least 184 over-expressed genes, as compared to cattle inoculated with the virulent *B. bigemina* strain in which only 19 over-expressed genes were identified. Among the DEG that encode proteins with a function in the bovine immune response, 33 DEG were over-expressed and 3 DEG were sub-regulated in cattle inoculated with the attenuatted strain (GII), as compared to cattle inoculated with the virulent *B. bigemina* strain (GI).

Conclusions

These data provide information on the possible molecular mechanisms involved in the induction/silencing of immune responses in cattle inoculated with each of the strains analyzed, providing molecular markers that could be correlates of immune protection or immune depression.

1366 - Controlling cryptosporidiosis with paromomycin sulfate as metaphylactic treatment in neonatal dairy calves

Author: Sonia Vazquez Flores, Stephany Barrera-Almanza, Fernando Sánchez Martínez

Objectives

The objective of the studies was to control cryptosporidiosis before its clinical presentation during the second week of life to diminish the consequences of loss of weight under different raising systems.

Material and methods

A total of 120 newborn female calves (F) were included in the studies from three different dairy private facilities. The study sites were Study 1 (S1) in northern México, Study 2 (S2) in central México, and Study 3 (S3) in western México. Calves were raised in individual crates and randomly selected at birth to one of the assigned treatments. Treatments were the following: control (C), where calves received the antibiotic treatment of choice in the dairy for scours, commercial prophylactic treatments vs *Cryptosporidium* (T1a and T1b), and paromomycin sulfate 50 mg/BW for 7 days (T2). S1 enrolled 20F in elevated crates with C and 20F with T2; S2 enrolled 21F in C, 14F in T1a, and 23F in T2. And S3 enrolled 30F in T1b, and 28F in T2. T1a was used on days 2 and 5 as indicated on the label, while T1b was used from day 2 of life with 7d of treatment. Personnel involved in the trials had previous training to detect loss of appetite as the first attitude change in calves where paromomycin sulfate treatment was given with or without scouring. Passive immunity was determined with Brix at 48h of age. A daily scoring system to record diarrhea was used for the first 60 days of life. Feces were collected at 7 and 14d of age, and diagnosed with a morphological analysis with acid-fast staining in commercial and research laboratories. Calves had their weight measured at birth, 60 (S2/S3), 90 (S1/S3), and 120 (S2). All calves received pasteurized colostrum, and the total population received pasteurized milk until 55d of their life. Feeding schemes were for S1: 5 times in 24 h, 20% of their body weight (BW), S2: twice a day, 20% of their BW; S3: twice a day 18% of their BW. Statistical significance was considered at $P<0.05$, data were analyzed using mixed procedures with JMP 17.1 and JASP 0.18.1. Treatments, site, and housing were the fixed effects, random effects were scours and appetite, while weight was covariate within the treatment.

Results

Results from passive immunity indicated that all sites were similar in Brix among all study groups. In the cumulative analysis of scours from the first week of life, S1 showed for C: 6.35±3.8, and **T2*: *4±4.1* (P <0.05)**; S2 for C: 0.43±1.2, T1a: 0.71±1.3, T2: 0.2±0.7; S3: no data (ND). For week 2 of life, S1 showed scours for C: 2.95± 3.4, and **T2*: *0.35± 0.8* (P <0.05)**; S2 for C: 6 ± 3.7, T1a: 9.71 ± 4.9 and **T2*: *2.2 ± 2.4* (P <0.000)**; and S3 for T1b: 9.6±4.5, **T2: *0.79±1.5** (P < 0.000)**. For appetite scoring S1 for C: 0.1± 0.31, and **T2*: *0 (P<0.001)***; S2 ND; S3 for T1b: 0.23±0.63, and **T2*: *0.18±0.48* (P<0.001)**. Cryptosporidium oocysts were in S1 at 7d for C: 30%, and T2: 40%, at 14 d for C: 35%, and T2: 20% (P =0.87); S2 at 7D for C: 42.9%, T1a: 39.3% and T2: 36.9%; at 14 d, C: 50%, T1a: 42.9%, and **T2*: *28.1%* (P<0.001)**; S3 at 7d for **T1b*: *0%* (P=0.003)**, and T2: 21.4%; at 14 d for T1b: 33% and **T2*: *14% (P=0.008)***. Weight at birth was similar in all study groups. Weight gain (Kg) for S2 at 60D was for C: 39.8±13.4, T1a 29.3±23.2, T2: 39.2±10.1. S3 for T1a: 30.2±12.9, **T2*: *37.1±5.6* (P=0.007)**. At 90d in S1 for C: 38.3±20.8, **T2*: *51.9±19.7***

(P<0.05), at 120d in S2 for C: 83.2±17.8, **T1a*: *65.3±16.9 (P= 0.006)***, T2: 82.1±16.2. Additionally, in S2, for pneumonia cases indicated that C: 0.33±0.5, T1a: 0.5±0.5, and **T2*:*0.17±0.4 (P>0.05)***.

Conclusions

In conclusion, paromomycin sulfate (T2) showed during pick of cryptosporidiosis: 34-50% less oocyst elimination allowing for local immunity with fewer clinical signs, no appetite loss, and fewer pneumonia cases. Weight gain was consistently higher in the T2 study group at 60, 90, and 120 days of age, when compared to prophylactic treatments to control *Cryptosporidium* spp. Good passive immunity, higher plains of milk feeding, and close follow-up of clinical cases contribute to cryptosporidiosis control, along with biosecurity measures and opportune treatment.

1374 - A mixed-methods study of the use of and producer perceptions about parasite control strategies implemented on cow-calf operations in Alberta, Canada

Author: Samuel Peter Agbese, John Gilleard, Caroline Ritter, Claire Windeyer

Objectives
Pharmaceutical interventions, pasture management, and biosecurity practices are some of the parasite control strategies implemented on cow-calf operations in western Canada. Currently, there is a lack of information regarding cow-calf producers' perceptions of on-farm parasite control strategies and their rationale for accepting or rejecting their adoption. The objective of this mixed-methods study was to describe producers' perceptions about on-farm parasite control practices and the strategies implemented on farm by those producers.
Material and methods
A questionnaire about parasite control strategies was delivered to 47 cow-calf producers who are Alberta residents and participants in the Canadian cow-calf surveillance network (C3SN). Subsequently, 15 respondents who indicated willingness to be interviewed were contacted via email to participate in a semi-structured telephone interview. Themes were extracted from the interview transcripts using thematic content analysis.
Results
The majority of questionnaire respondents (96.8%) indicated that they typically use parasite control products (i.e., parasiticides), followed by pasture management (64.5%), biosecurity (22.6%), and other strategies (16.1%) when attempting to control parasites. Respondents rated the importance of various factors when selecting these products, with effectiveness against external parasites, effectiveness against internal parasites, and ease of application mostly rated as highly important, and price rated as less important. Thematic content analysis of interview transcripts revealed factors that impacted participants' rationale for the implementation of parasite control strategies included veterinarians' influence, drug effectiveness, ease of application of drugs, knowledge about the practice, risk aversion or acceptance, access to research, trust, concerns about other infectious diseases, considerations about the land, personal experience, and weather factors.
Conclusions
The result of the mixed-methods study may improve future research efforts by helping to tailor them to the on-farm realities and preferences of producers. Identified barriers to parasite control strategies may be addressed through research, extension, or policy to encourage the adoption of recommended strategies that could improve parasite control. Producers' access to information and the right parasite control products, information regarding refugia including more research on how to effectively carry out refugia-based practices in cattle, education on how pasture management helps control parasites, and improved veterinarian-producer communication were identified as areas that could be addressed to improve the application of parasite control strategies in the future.

1389 - Evaluation of the effect of paromomycin dosed at 150 mg/kg on commensal gut microbiota of healthy calves

Author: Pascal Butty, Anne Trotel, Damien Achard, Juan-Munoz Bielsa, Yves Jacquemet, Laetitia Hernandez, Fatima M. Zali, Pedro Pinho

Objectives

The objective of this study was to assess the impact of paromomycin administered orally for 5 days, on the development of resistance in intestinal commensal flora of healthy calves.

Material and methods

Twenty-five healthy calves originating from 7 different French farms and aged 12-21 days at the start of the study were enrolled. Calves were housed collectively, fed with milk replacers twice a day and randomly allocated to treatment (Gabbrovet Multi®, Ceva Santé Animale, 150 mg/kg once a day for 5 days; n=23) or untreated control group (n=2) on Day 0. Calves' health was monitored daily based on faeces status, depression score, and appetite (0-2 scorings). At Day+37, calves were euthanized. Faecal samples before, during and after treatment with paromomycin were collected to trace the evolution of paromomycin susceptibility of commensal intestinal *Escherichia coli.* For this, fresh faeces of the animals were collected at Day-1, Day+4, Day+20 and Day+36 in a sterile 50 ml sample container then immediately stored at -80°C and later transported frozen to a microbiology laboratory for isolation and purification of commensal *E. coli* strains. Twenty purified and randomly isolated colonies of *E. coli* were recovered from every faecal sample of each collection time point for further microbiological analysis. Because of the very high number of strains, clusters of epidemiologically related strains were identified by mean of mass spectrometry (MaldiTof Biotyper Compass explorer software), then subjected to paromomycin MIC determination by mean of a customized microdilution approach (UMIC) and an aminoglycoside antibiogram in accordance with CLSI guidelines. Based on the CA-SFM breakpoint of kanamycin for Enterobacteriaceae, the evolution of resistance to paromomycin of the *E. coli* strains was monitored for every strain/sample by comparing the results obtained individually for each animal at different times during the study with those obtained prior to paromomycin administration.

Results

A total number of 1780 *E. coli* were isolated for further individual analysis. Before any treatment administration (Day-1), the commensal *E. coli* flora consisted in both susceptible clones (69% observed in 16 out of 23 calves) and clones already exhibiting resistance (31% observed in 13 out of 23 calves) to paromomycin. Treatment with paromomycin did not result in the development of resistance in primarily susceptible *E. coli*. Indeed, during this study, no isolated clones identified before or after treatment have acquired paromomycin resistance. In fact, paromomycin treatment eliminated a high proportion of the susceptible commensal *E. coli*, thus allowing pre-existing resistant commensal *E. coli* and pathogenic *E. coli* to take over and predominate in the intestinal microbiota. This rearrangement was transitory, and the selection pressure exerted by paromomycin to select resistant clones strongly decreased after the end of treatment between Day+20 and Day+36. This was indeed demonstrated by a significant reappearance of the commensal *E. coli* clones susceptible to paromomycin (40 % at Day+36 compared to 7 % at Day+20; 10 % at Day+4 and 69 % at Day-1, respectively).

Conclusions

During this study, no acquisition of resistance at the clonal level was observed in *E. coli* following paromomycin treatment. The susceptibility profile tracked for each clone of *E. coli* did not show any shift of the MIC between the sampling points. The treatment as expected disrupted the flora by preferentially selecting preexisting resistant clones capable of surviving. A gradual reversion to the initial conditions of the colibacillary flora was observed after paromomycin treatment discontinuation. The curative bactericidal high dosage of paromomycin used in this study has certainly contributed in preserving the strains from acquiring resistance. Particular attention should be taken when lower dosage regimen is used (*i.e.* prophylactic usage) as these have been documented for inducing more readily resistance.

1391 - Systematic review of the effectiveness of pharmaceutical interventions to treat or control gastrointestinal nematodes in pre-weaned beef calves on cow-calf operations

Author: Samuel Peter Agbese, Margarita Sanguinetti, Allison Vesely, John Gilleard, Caroline Ritter, Heather Ganshorn, Claire Windeyer

Objectives
Gastrointestinal nematode (GIN) infections in pre-weaned beef calves can lead to significant economic losses for cow-calf operations. Pharmaceutical interventions are commonly used to treat or control these infections, but there is a lack of information regarding the current scientific knowledge that summarizes the effectiveness of these interventions in controlling GIN in pre-weaned beef calves. Therefore, the objective of this study was to compile and assess the current scientific literature on the effectiveness of pharmaceutical interventions to treat or control GIN in pre-weaned beef calves on cow-calf operations.
Material and methods
A systematic review was conducted using the following databases: MEDLINE on the Ovid platform (including PubMed), Web of Science, CAB Abstracts, Scopus, and ProQuest Dissertations. Screening was conducted using Covidence software (Veritas Health Innovation, Melbourne, Australia). Title and abstract followed by full-text screening were each conducted by 2 independent reviewers, with a third providing input when needed. Information about the bibliographic data, study design, population characteristics, pharmaceutical intervention (i.e., anthelmintic drug, route, dosage), and outcomes of interest (i.e., fecal egg count: FEC; fecal egg count reduction test: FECRT; GIN genera) were collected. Risk of bias was assessed based on the Cochrane Review Handbook.
Results
The final database was composed of 29 articles published between 1970 and 2021. The most common drugs examined in the included studies were fenbendazole of the benzimidazole class of drugs, ivermectin of the macrocyclic lactones, and morantel tartrate. Regarding the route of administration, oral drenching was most often studied, followed by injectables, then boluses, pour-ons, and deworming blocks. The main GIN genera identified pre- and post-treatment with an anthelmintic drug were Ostertagia spp., Trichostrongylus spp., Cooperia spp., Haemonchus spp., Oesophagostomum spp., and Nematodirus spp. Effectiveness, measured as a reduction in FEC based on the FECRT, was assessed in 5 articles. Studies investigating drugs in the benzimidazole class of drugs all found FECRT above 96%. In contrast, the studies that examined drugs in the macrocyclic lactone class reported FECRT between 15 and 93%.
Conclusions
Overall, there is evidence that the administration of the anthelmintics assessed in the presented studies resulted in calves having statistically significantly lower FEC compared to untreated controls following treatment, indicating some effectiveness of the anthelmintic interventions. Furthermore, there is some available evidence that the macrocyclic lactone class of drugs appeared to have less effectiveness than the benzimidazole class of drugs in controlling GIN in pre-weaned beef calves, as demonstrated by FECRT. However, more high-quality studies are needed to expand the

existing knowledge. This study provides summary of the existing evidence on the effectiveness of anthelmintic drugs for better informed decisions and improved strategies for GIN control in pre-weaned beef calves.

1427 - Effect of cow's parity order and its antibody level at gestation on the humoral immunity of calves for cattle anaplasmosis

Author: Jose Reck

Objectives

Anaplasmosis is a widely distributed vector-borne disease of cattle caused by the bacteria *Anaplasma marginale*, which may lead to severe losses in beef and dairy cattle production. Anecdotal information among farmers suggested that some cows may deliver calves more resilient to anaplasmosis. To investigate this, we tested two hypotheses: (i) whether the parity order or (ii) cow antibody levels may influence the humoral immunity of pre-weaning calves against cattle anaplasmosis.

Material and methods

The study was conducted in one farm located in Southern Brazil, Uruguaiana municipality, Rio Grande do Sul state (RS). For this study, we sampled 170 cattle (*Bos taurus taurus*, Angus breed) on a farm in Southern Brazil, comprising 85 cows (50 multiparous and 35 primiparous) and their 85 calves (50 days old). The multiparous cows consisted of 3rd and 4th parity order cows (average age of 55 months old, minimum 48, maximum 84). The primiparous cows showed an average age of 24 months old (minimum 18, maximum 30). The 85 calves included in the study were the offspring from the cows described above, and at the sampling time showed an average of 50 days old (minimum 40, maximum 70 days). Blood samples of all cows were taken at the same time, during their last trimester of gestation (October, early spring). In the same way, blood samples of all calves were collected at the same time (December, late spring), with animals showing an average of 50 days old. For the detection of antibodies against *A. marginale*, an indirect enzyme-linked immunosorbent assay (iELISA) was used.

Results

Out of the 170 animals tested, 136 (80%) were considered seropositive for the presence of antibodies against *A. marginale*. All the cows (100%) were seropositive for *A. marginale*, compared to just 51 out of 85 (60%) of the 50-days old calves. When considering the calves from primiparous and multiparous cows, there was no statistical difference in the seroprevalence for *A. marginale*. We used the OD value of each animal in the iELISA assay as an indicator of their antibody levels against *A. marginale*. When the OD values of the two groups of calves originating from cows with different parity orders were compared, the antibody levels of calves from the primiparous cows (1st parity order) were significantly lower than the antibody levels observed in those originating from multiparous cows ($P < 0.05$). To further explore humoral immunity against *A. marginale*, a threshold was used to classify cows as those "with high antibody levels" and "with low antibody levels". Indeed, "calves from cows with high antibody levels" also showed significantly higher OD values, i.e., high antibody levels against *A. marginale*, than those born from mothers with "low antibody levels" ($P < 0.05$). Interestingly, considering the number of calves with antibodies against *A. marginale*, the proportion of seropositives was higher in "calves from cows with high antibody levels" (79%) than in "calves from cows with low antibody levels" (45%). Remarkably, an odds ratio (OR) analysis suggests that "calves from cows with a high antibody level" showed approximately four times greater odds of being seropositive for *A. marginale* at 50 days old than those born from mothers with a "low antibody level".

Conclusions

The set of results presented here allows us to conclude that the calf's antibody level against *A. marginale* is related to the cow's parity order, and that the odds of a calf showing passive humoral immunity against *A. marginale* in the pre-weaning phase are related to the mother's antibody level. Thus, in the future, and after validation in other conditions, it is possible that cow serology may be used as an indicator of risk for anaplasmosis in calves. Also, the findings reported here improve the understanding of the *A. marginale*-cattle relationship, particularly regarding maternal immunity.

1432 - Risk Factors Associated to Protostrongylidae Infection in Small Ruminants from Centre Region of Portugal

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Objectives

Small ruminant production in Centre region of Portugal is essentially conducted in a semi-extensive husbandry system, exposing animals to parasitic infections, which can negatively impact the production. *Dictyocaulus filaria* and several species of the family Protostrongylidae can infect ovine and caprine, causing generally subclinical infection or mild respiratory clinical signs. To our knowledge, no reports have been published on lungworm infection in small ruminants in Portugal. Thus, this study aimed to estimate the prevalence of lungworm infection and identify risk factors to define appropriate control measures.

Material and methods

Faecal samples of 203 goats and 208 sheep from 30 herds, located in three districts of Centre region of Portugal were collected *per rectum* and subjected to modified Baermann test. First stage larvae (L1) were morphologically identified under optical microscopy. Background information from herds and parasitological results were subjected to descriptive and inferential statistical analysis. Binary logistic regression (method Forward conditional) was used to identify risk factors associated to Protostrongylidae infection

Results

The overall prevalence of infection was 57.7% (95% CI: 0.525-0.624), significantly higher in goats (95.6%; 0.921-0.978) than in sheep (20.7%; 0.156-0.266) ($p<0.001$). All animals were infected by species of the Protostrongylidae family, except three sheep that presented *D. filaria* simple infection. The proportion of Protostrongylidae infected animals that did not share pasture (72.2%) was significantly higher than those that share pasture (12.4%) ($p<0.001$). Infection is most frequent in animals dewormed once a year (80.2%) than twice a year (21.5%) ($p<0.001$). The proportion of infected animals was also statistically different according to the antihelmintic compound used (eprinomectin 48.3%; albendazole 92.5%; mebendazole plus closantel 34.7% and ivermectin plus clorsulon 85.5%) ($p<0.001$). Binary logistic regression confirmed these results, indicating that the risk of Protostrongylidae infection was low in sheep (OR=0.136; p=0,136) compared with goats. Furthermore, the risk of infection for animals that do not share pastures was 3.755 (p=0.017) times higher than for those that share pastures. Animals dewormed annually presented a risk of infection 12.316 (p=0.001) times higher than those dewormed every six months. Animals dewormed with albendazol, mebendazol plus closantel or ivermectin plus clorsulon presented a risk of infection 0.016 ($p<0.001$), 0.057 (p=0.006) or 0.223 (p=0.052) times lower than those dewormed with eprinomectin.

Conclusions

The risk of Protostrongylidae infection was slightly higher in sheep than in goats, as frequently documented. Sharing pastures appears to be a protective factor, probably by

increasing the extent of available pasture, which dilute its contamination with infective terrestrial molluscs. The frequency of deworming has a greater protective effect than the anthelmintic compound used on Protostrongylidae infection, pointing to the importance of biannual deworming in controlling lung parasite infection.

Acknowledgments: This work was supported by National Funds through FCT – Foundation for Science and Technology, within the scope of the GHTM projects UID/04413/2020, CITAB UIDB/04033/2020 and CERNAS UIDB/00681/2020, as well as through the Projeto de Ignição e Provas de Conceito INOVC+ (PIPC/003/2022_02) cofounded by Centro 2020, Portugal 2020 and EU through IPV.

1466 - RECOGNITION OF TWO SERINE PROTEASE PEPTIDES (S28) DERIVED FROM Haemonchus SPP BY IgG OF INFECTED LAMBS AND CALVES

Author: Ma. Eugenia López Arellano, René Camas-Pereyra, Génesis A. Bautista-García, David E. Reyes-Guerrero, Jocelyn Maza-Lopez

Objectives

Among the main nematodes that affect ruminants in Mexico, *Haemonchus* spp. is considered highly pathogenic for ruminants, causing anemia, weakness and death, mainly in young animals. The aim of this study was to analyze sera from sheep and cattle infected by gastrointestinal nematodes (GIN) to evaluate the recognition of two-serine protease peptide sequences (S28) derived from *Haemonchus* spp.

Material and methods

This work was carried out at the National Center for Disciplinary Research in Animal Health and Safety (CENID-SAI), INIFAP in Jiutepec, Morelos, Mexico. A volume of 6 ml of blood sample was collected from 6 lambs of 4 months of age (Pelibuey) and 6 calves of 6 months of age (Swiss American x Indu Brasil). Three out of them, sheep and cattle, were used as negative controls. The positive infection for GIN in ruminants was confirmed by coproparasitological tests and traditional PCR to identify the nematode specie such as *Haemonchus* spp. (Reyes-Guerrero et al. 2020).

An indirect enzyme-linked immunosorbent assay (iELISA) was performed where Flat-bottom ELISA microplates were used for the fixation of mixed S28 peptides at 0.25 µg/ml. The remaining iELISA methods have been described previously (Camas-Pereyra et al. 2023). An analysis of variance (ANOVA) and a multiple comparison of means using the Dunnett test ($p < 0.05$) were performed to determine the difference between naturally infected ruminants with respect to negative controls, using the IBM® SPSS® STATISTICS statistical package.

Results

All animals were positive for NGI and *Haemonchus* spp. From the sera analyzed, the group of lambs showed the highest absorbance (1.11±0.0143 Abs) compared to negative control ($p < 0.05$) showing fecal egg counts (FEC) more than 2000. Although lower Abs and FEC values (0.66±0.0071) were observed in infected calves with GIN, differences were also statistically significant compared to negative controls ($p < 0.05$

Conclusions

The S28 peptide mixture used in the present study appeared to have potential to be included in methods of diagnostic as biomarker in infected ruminants with *Haemonchus* spp and other GIN. Furthermore, the IgG level to recognize the mixture of both S28 peptides suggests immunogenic activity during natural nematode infection

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1467 - GENE EXPRESSION OF Hco-Pgp16 ASSOCIATED TO THE SUBSTRATES COMPETENCY IN Haemonchus contortus (xL3) RESISTANT TO IVERMECTIN

Author: David E. Reyes-Guerrero, Ma. Eugenia López Arellano, Jocelyn Maza-Lopez, René Camas-Pereyra, Pedro Mendoza-de-Gives

Objectives

Haemonchus contortus is the most important parasitic nematode in tropical regions. The main control method available for the control of *H. contortus* is anthelmintic drugs (AH). However, constant exposure to them has generated anthelmintic resistance (AR). The coding genes of the P-glycoprotein transporter proteins (P-gp) and the increase of their gene expression levels have been associated with the development of resistance to macrocyclic lactones (LM) such as ivermectin (IVM). The aim of this work was to perform the analysis of gene expression of Hco-pgp-16 from larvae in transition (xL_3) of *H. contortus* resistant to ivermectin (IVM), which were exposed to IVM, verapamil (VER) and a curcumin dispersion (CUR) *in vitro* tests, as well as their respective combinations (VER/IVM and CUR/IVM) in order to evaluate the competence of substrates for Hco-pgp16 in *H. contortus* resistant to IVM and reduce the resistance problem

Material and methods

Development of xL_3 was performed in culture bottles with Hank's Balanced Salts medium supplemented with 0.02% ovine erythrocytes. The larvae were incubated for 21 days at 37°C with 5% CO2 atmosphere. IVM, analytical grade was used in six different concentrations (0.078, 0.1562, 0.3125, 0.625, 1.00 and 1.25 mg/mL). Also, a dispersion of CUR and VER analytical grade, were used at a concentration of 0.078 mg/mL. Each IVM concentration under study was combined with the concentration of 0.078 mg/mL of CUR and VER, as the case was. Three replicates were made for each treatment at a time interval of 48 hours, using microtitration plates and 100 xL3. Performed the count of dead and live larvae from each well. On the other hand, RNA extraction was performed from 10,000 xL3, used in IVM treatments and combinations of IVM/CUR and IVM/VER, only at the concentration of 1.25 mg/mL. RNA was purified with chloroform and isopropanol, precipitated with 75% ethanol. Subsequently, cDNA synthesis was performed in triplicate by commercial kit from 300 ng of total RNA extracted per sample. qPCR reactions were performed using the Hco-pgp16 and GAPDH and Beta-tubulin genes as constituents. The relative expression analysis of the Hco-pgp16 gene was calculated with the ΔΔCt method based on the threshold cycle value (Ct) per gene. The data analysis was performed on the Qiagen GeneGlobe Data Analysis Center web platform. (https://geneglobe.qiagen.com/us/analyze/). Untreated xL_3 were used as the control group for relative expression analysis.

Results

A mortality rate of 37% was shown for CUR and VER (controls) at 0.078 mg/mL. In the IVM concentrations evaluated, larval mortality percentages were observed between 40 and 45%. Regarding the combination between CUR/IVM, between 41 and 50% of mortality was determined, obtaining the highest percentage in the concentration of 1.25 mg/mL of IVM ($p \leq 0.05$). In the combination between VER/IVM, an increase in mortality of xL_3 was also observed (between 44 and 51%), showing significant differences at the same IVM concentration ($p \leq 0.05$). Increased relative expression levels of the Hco-pgp-16 gene were observed in xL_3 treated with VER only, obtaining a value of 2.35 fold

change higher than the control group. In contrast, the Hco-pgp-16 gene in xL_3 treated with CUR showed normalized expression values (0.85). On the other hand, decreased relative expression of the gene under study was detected in treatments with IVM, CUR/IVM and VER/IVM, obtaining expression values of 0.09, 0.08 and 0.14 fold less respectively, compared to the control group and the housekeeping genes used, GAPDH and β-tubulin

Conclusions

These results suggest that the Hco-pgp-16 gene may be involved in anthelmintic detoxification mechanisms, because here it is shown that the relative expression values depend on the exposure of the Hco-pgp-16 protein to the different combinations and competencies of substrates, so these findings could contribute to the development of new control alternatives that help maintain the efficacy of AH against parasitic diseases in the field.

1468 - Observations of IgG response to recognize a Glutathion-S Transferase peptide related to Haemonchus placei infection

Author: Jocelyn Maza-Lopez, Ma. Eugenia López Arellano, David E. Reyes-Guerrero, René Camas-Pereyra, Rosa I Higuera-Piedrahita, Vìctor H. Bermúdez-Morales

Objectives

Haemonchus spp or barber's pole worm is a high pathogenic nematode of ruminants due to its hematophagous habits. This nematode is mainly localized on tropical and template regions affecting young cattle. The use of anthelmintic drugs is the only commercial method of control against parasitic nematodes of ruminants. However, problems of anthelmintic resistance are affecting small and big ruminants. Previous studies looking for other strategies of control such as the host-immune response suggest that the Glutathione S-Transferase (GST) family as important protein group to regulate the nematode infection. Therefore, the objective of this study was to analyze the recognition of a GST peptide design from the Haemonchus spp transcriptome by IgG in calves infected with *Haemonchus placei* to contribute in the development a possible immunized agent

Material and methods

Two GST´s sequences from the transcriptome of a Mexican *H. contortus* isolate was downloaded and analysed to select and design an immunodominant peptide using bioinformatics programs (BioProject: PRJNA877658; Reyes-Guerrero et al., 2023). To analyse the recognition of a GST peptide, seven calves were orally infected with 50,000 infective larvae of *H. placei* and the nematode infections was monitoring through 11 weeks by the number of eggs per gram (EPG) of feces and the package cell volume (%PCV). Blood-samples from infected calves were collected to estimate the level of IgG antibody to recognize the GST peptide for 11 weeks using the indirect enzyme-linked immunosorbent assay (ELISA) and samples from a negative control group.

Results

The mean of EPG and %PCV for 11 weeks from infected calves with *H. placei* showed 260 ± 43.72 of EPG and 28.57 ± 0.71 respectively. The GST peptide were recognized by IgG of infected hosts with *H. placei*, displaying an optical density (OD) of 0.52 ± 0.06, compared to the control groups with a mean of 0.38 ± 0.07 OD ($p < 0.05$). In addition, infected calves with *H. placei* with high number of EPG showed low level of IgG. In contrast, infected host with low number of EPG had high IgG level, indicating different active response of the GST peptide to both groups.

Conclusions

In the present work, we identify the recognition of a peptide derived from the GST sequences derivate from the Mexican transcriptome of *Haemonchus contortus* by IgG of infected calves with the other species the genus *Haemonchus*, *H. placei*, indicating the importance of its biological function and conservation of the GST protein during the development of the parasite, therefore Its study may contribute to the control and diagnosis of this nematodosis.

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as a Tool for the Knowledge of Ivermectin Resistance Mechanisms. Pathogens, 12(3),499. https://doi.org/10.3390/pathogens12030499

1474 - Observational longitudinal study on Toxoplasma gondii infection in beef cattle: serological and haematological findings

Author: Filippo M. Dini,Joana G.P. Jacinto, Damiano Cavallini, Andrea Beltrame, Flavia S. Del Re, Laura Abram, Arcangelo Gentile, Roberta Galuppi

Objectives

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is a globally distributed zoonotic infection with significant implications for human and animal health. This study aimed to investigate the dynamic of the prevalence of *T. gondii* infection in a population of beef cattle at three different stages of their productive lifespan and to examine the impact of *T. gondii* serological status on blood parameters.

Material and methods

The study was conducted within a commercial fattening unit in the province of Modena, Italy with Limousine bulls imported from France. A total of 264 animals were delivered to the fattening unit in weekly shipments organized in numerically diverse groups, spanning six consecutive weeks. These animals originated from various farms across France, encompassing different regions within the country. Most of these bulls were primarily raised either on pastures or in indoor free stall systems with straw bedding. Upon their entry into the fattening unit, the bulls were approximately 11 months old and had an average weight of 400 kg. No quarantine period was performed. At the arrival, animals were randomly allocated in pens of 6 bulls and fed an adaptation diet to reduce dietary stressors. The production cycle lasted between 5 to 6 months. A biosecurity assessment was performed at arrival to the unit (T0) and 15 days after arrival (T1). Blood samples from 88 animals were collected for haematological investigation at T0 and T1. Two animals were randomly selected from each pen at T0, and the same subjects were again sampled at T1. Five months after T0, cardiac blood samples were obtained at the slaughterhouse (T2), from 56 of the 88 animals that underwent a blood sampling at T0 and T1. Blood collection took place during the heart excision process, where approximately 10-40 ml of blood was collected in a 50 ml falcon tube and kept at room temperature until further processing. *Toxoplasma*-specific serological testing using indirect fluorescent antibody tests (IFAT) was performed on the blood samples obtained from T0 to T2. Descriptive statistics were generated. For continuous variables, normality was tested by the Shapiro-Wilk test and non-normally distributed variables were Box-Cox transformed before the analysis. The evaluation of differences between the positive/negative to *T. gondii* and different IFAT titres was undertaken using the Mixed Model Procedure. Principal component analysis (PCA) was used to reduce the variables to factors.

Results

The biosecurity assessment did not vary between T0 and T1 being scored as medium with a 61%. Results revealed a dynamic pattern of *T. gondii* seropositivity in cattle, with an initial prevalence of 30.6% at arrival (T0) that increased to 44.6% at 14 days (T1) and then decreased slightly to 39.3% at slaughter (T2). The distribution of IFAT titres in the seropositive group for *T. gondii* were as follow: at T0 51.2% (1:80) followed by 40.7% (1:40) and 7.4% (1:160) as the maximum titre; at T1, the animals with a titre of 1:40 slightly increased reaching the 43.2% followed by the ones with a titre of 1:160 (29.7%), 1:80 (16.2%), 1:320 (8.1%) and finally 1:1280 (2.7%); finally at T2, the percentage of

animals with a titre of 1:40 increased again as the most common category (54.6%), followed by animals with titres >1:80. Seroconversion occurred in 13 animals (14.6%) from T0 to T1, and in 5 (6%) from T1 to T2. No animals lost detectable antibody titre from T0 to T1, while at T2, 12 (14.5%) bulls previously positive tested negative for IgG. Interestingly, seroconversion was observed during the study, indicating ongoing infections, and antibody waning occurred in some animals. In terms of blood parameters, seropositive cattle exhibited significantly lower mean corpuscular volume (MCV) and a higher neutrophil-lymphocyte (N/L) ratio, suggesting an activation of the innate immune response. Furthermore, cattle with higher antibody titers displayed higher neutrophil counts.

Conclusions

This study provides a longitudinal investigation on the serological status for *T. gondii* in naturally exposed beef cattle. These findings provide valuable insights into the clinico-pathological aspects of natural *T. gondii* exposure in cattle and underscore the importance of monitoring and managing *T. gondii* infection in livestock production systems.

1477 - IgY antibodies to significantly reduce cryptosporidium infection: efficacy study in an experimentally infected calf model

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Objectives

Cryptosporidium parvum is a zoonotic protozoan parasite that causes diarrhea in neonatal calves resulting in significant economic losses. *Cryptosporidium parvum* is transmitted through the fecal-oral route, and the severity of the disease depends on the immune status of the animal (Pumipuntu *et al*., 2018). In Argentina, *C. parvum* is the major enteric pathogen (de Alba *et al*., 2023) and there are no commercially available vaccines or effective treatments to control the impact of the disease. Avian IgY antibodies have been shown to confer passive immunity to viral diseases such as those caused by rotavirus (Vega *et al*., 2020) and coronavirus (Bok *et al*., 2023). The objective of this study was to develop a product based on *Cryptosporidium*-specific IgY antibodies and to evaluate its protection against infection and diarrhea in calves experimentally challenged with *C. parvum*.

Material and methods

Fifty white Leghorn laying hens were immunized intramuscularly with four doses each of 14 ug of recombinant *C. parvum* p23 protein produced in the baculovirus system. Whole eggs were collected and spray-dried. Eight calves were enrolled after birth, fed 2L of commercial colostrum (Calostro 100, SCCL), and inoculated orally with 6 million *C. parvum* oocysts at 2-5 days of age. Four of the eight calves (n=4) were treated with 2L of commercial whole milk supplemented with 20 g of the p23-IgY product, corresponding to a final titer of 1024 of p23-IgY in milk, twice daily for 14 days starting the day before inoculation. The remaining control calves (n=4) received 2L of milk without any supplementation, twice daily. Fecal samples were collected, and fecal scores (0-3) were recorded for 21 days after inoculation. Quantification of excreted oocysts was done by qPCR (18S gene).

Results

The average p23-specific IgY antibody titer achieved in chicken serum after four immunizations was 16,384. Fifteen kg of powder/product was obtained with a p23-IgY titer of 1024 (10 mg/ml). All eight calves that had been inoculated orally with *C. parvum* oocysts shed oocysts and developed diarrhea. Importantly, IgY treatment significantly reduced the duration of diarrhea and oocyst shedding compared to the control group (7.5 days vs 9.5 days and 2 days vs 9.25 days, respectively).

Conclusions

Since all inoculated calves developed diarrhea, we can assume that the infection model used is successful in mimicking natural disease conditions. Given the fact that the anti-p23 antibody titers obtained were significantly high, recombinantly expressed p23 is an excellent candidate for the development of parasite-specific IgY antibodies in chickens. Furthermore, the presented results show that IgY treatment is a promising tool to control *C. parvum*-associated diarrhea in neonatal calves. Finally, this is the first study that evaluates the efficacy of an IgY-based product to control *C. parvum* diarrhea in neonatal calves.

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1478 - Current Seroprevalence Status of Neospora bovis in Dairy Herds in the Province of Lima, Peru

Author: Luis Felipe Ruiz García, Rocío Silvia Sandoval Monzón

Objectives
To establish the current seroprevalence status of Neospora bovis in dairy herds in the province of Lima, Peru.
Material and methods
This observational and cross-sectional study, with a quantitative approach, was conducted between May 2021 and April 2022 in the primary commercial dairy herds of the province of Lima. Fifteen herds were selected, representing approximately 63% of the total population of commercial herds with more than 20 heads. Determination of the current status of Bovine Neosporosis in herds was based on random sampling within each herd, calculating a sample size of approximately 340 animals. A blood sample of 5 ml per animal was obtained using Vacutainer needles, and seropositivity tests for Neospora bovis were performed using a commercial kit. Data were analyzed using various statistical techniques to estimate prevalences and identify associations with the age of the animals.
Results
Regarding the collected data, out of a total of 342 sampled cows in 15 farms, the overall prevalence of Neospora bovis in the farms of the province of Lima was 48.98%, with a 95% confidence interval of ± 5.30%. It was also found that 100% of the sampled herds had at least one infected animal. Additionally, a comprehensive analysis segmented by age groups revealed specific prevalences for each of them. The results were as follows: 45.19% ± 13.53% for the group aged 6 months to 1 year, 45.31% ± 9.96% for the group aged 1 to 2 years, 44.09% ± 9.28% for the group aged 2 to 3 years, 58.04% ± 12.93% for the group aged 3 to 4 years, 79.55% ± 16.86% for the group aged 4 to 5 years, and 33.33% ± 37.72% for the group aged over 6 years. These findings provide a detailed insight into specific prevalences in each age range. The 95% confidence intervals for these prevalences in different age groups indicate certain levels of variability among prevalences in different age groups. The highest prevalence was observed in the 4 to 5-year-old group, with 79.55%. These results suggest a widespread presence of Neospora bovis in dairy herds in the province of Lima. In a recently published meta-analysis, it was found that in the South American region, the seroprevalence of this parasite was 18% (Ribeiro et al., 2019). Therefore, the findings of this study raise serious concerns regarding cattle health in the region. It is important to note that dairy cattle have 1.6 times more risk of this disease compared to beef cattle (Ribeiro et al., 2019). It becomes imperative to implement highly specialized management and control measures to mitigate the spread of bovine neosporosis in these significant dairy herds. The high observed prevalence underscores the urgency of an effective intervention to safeguard the health and productivity of cattle in this area, emphasizing the need for a proactive and strategic approach to address this concerning issue (Serrano et al., 2019).

Conclusions:
A high seroprevalence of Neospora bovis was found in the farms of the province of Lima (48.98% ± 5.30%), indicating a significant concern for cattle health in the region.

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1485 - Effect of a eubiotic on the frequency of neonatal diarrhea, fecal oocyst shedding and mortality in dairy calves naturally infected with Cryptosporidium spp

Author: Darío Caffarena, Joaquín Armendano, Mariana Bustos, Javier Sabbia, Caroline da Silva Silveira

Objectives

Cryptosporidium spp. is one of the main causes of neonatal calf diarrhea and mortality in neonatal dairy calves worldwide. Despite its negative impact, the availability of effective commercial products against this pathogen are still limited. The aim of this study was to evaluate the effect of a eubiotic, composed of phytochemicals and essential oils, on the frequency of diarrhea, fecal oocyst shedding and mortality in naturally infected dairy calves with *Cryptosporidium* spp. in a commercial dairy farm.

Material and methods

The study was performed in a commercial dairy farm located in Campana, Colonia, Uruguay. A total of 72 newborn calves, were assigned to a randomized, double-blind experimental design with two treatments of 36 animals each. Treatment A received an eubiotic and treatment B (control/placebo) received a 0.9% sodium chloride solution (NaCl 0.9%; control/placebo group - B). Both treatments were administered orally from the first day of life until weaning at 9 weeks of age (day 1 to 3: 25 mL/animal/day; day 4 to weaning: 10 mL/animal/day). Within 48 hours after birth, a serum sample was taken from each animal to indirectly assess passive immunity transfer (PIT) using a refractometer. Fecal samples were collected biweekly during the first 5 weeks of life to evaluate the frequency of diarrhea by fecal score and fecal oocyst shedding by semiquantification. Additionally, seven pools of diarrheic feces (defined as score ≥2 on a scale from 0 to 3) were analyzed to assess the presence of Rotavirus Group A, bovine Coronavirus, *Escherichia coli* F5+, and *Cryptosporidium* spp. by lateral flow immunochromatography test, and *Salmonella* spp. by culture. To evaluate the treatment's effect on diarrhea frequency, oocyst shedding, and mortality, generalized linear mixed models (GLMM) were applied. The models were adjusted by the baseline measurement of the outcome (when available) using penalized cubic splines. For oocyst shedding, the risk of an animal shedding oocyst was modelled first and secondly, if there was a difference in the oocyst count between treatments. A significance level (alpha) of 5% ($p < 0.05$) was considered for all the statistical analyses.

Results

Regarding PIT, the median for treatment A was 9.45 °Brix (IQR: 8.7 – 10.6) and for treatment B 9.65 °B (IQR: 8.8 – 10.7), without significant difference (p=0.536). The only enteropathogen detected in diarrheic feces was *Cryptosporidium* spp. and 97.2% of the animals were positive by semiquantification. There was no growth of *Salmonella* spp in the bacterial cultures. In both treatments there was an increase in diarrhea frequency in the 3rd and 4th sampling (2nd week of life) with no effect of the treatments on the diarrhea frequency (p=0.3484). Concerning oocyst shedding, animals in treatment A had a mean (±SD) of 0.56 ± 2.52 and 0.22 ± 0.96 in treatment B. Similarly to diarrhea frequency, there was no treatment effect on the risk of shedding nor semiquantification of oocyst (p=0.1899 and p=0.8324, respectively). During the trial, 5 animals died (6.9%

overall mortality). Of those animals, 3 were from treatment A [%, mean (standard error): 8.3 (6.8)] and 2 from B [5.6 (2.3)]. There was no treatment effect on mortality (p=0.645).

Conclusions

The results showed that, under the conditions of the present trial, the eubiotic had no significant effect on the frequency of diarrhea, oocyst shedding, or mortality. It is important to emphasize that this study provides a basis for future research and the development of alternatives for *Cryptosporidium* spp control in Uruguay. These investigations should be conducted under field conditions and following rigorous scientific methods to generate solid and relevant information for end-users**.**

1496 - Bio-guided isolation of an anthelmintic compound from Artemisia cina against Haemonchus contortus L3

Author: Luis David Arango-De la Pava, Manasés Gonzaléz-Cortazar, Raquel López-Arellano, Jorge Alfredo Cuéllar-Ordaz, Alejandro Zamilpa, Rosa Isabel Higuera-Piedrahita

Objectives

Determine the presence of new secondary metabolites from *Artemisia cina* with anthelmintic effects against *Haemonchus contortus* L_3.

Material and methods

Fresh pre-flowering leaves and stems of *A. cina* were obtained from [Hunab], which harvests the plant in a greenhouse. The plant was air-dried under dark conditions. This plant material was ground and extracted with methanol, ethyl acetate, and n-hexane by maceration at room temperature for 48 hours. The liquid extract was filtered (No. 4 Whatman filter paper) and concentrated to dryness at 40 °C under low pressure. The extract was finally lyophilized and kept at 4 °C for phytochemical and biological assays. The larval mortality effect of the extracts, fractions, and isolated compounds was performed in vitro in a 96-well microplate, using approximately 100 L_3 larvae per well. A bio-guided chromatographic separation of the extract with the highest activity was carried out to determine the compound responsible for the larval mortality effect of the extract. This extract was separated through open column chromatography, using normal silica gel 60 (Merck, 0.015-0.040 mm) as the stationary phase and ethyl acetate-n-hexane as the solvent gradient system, monitored with thin-layer chromatography. The isolated compound was identified through HPLC-DAD, UPLC-MS, and one- and two-dimensional Nuclear Magnetic Resonance (NMR) experiments (^{1}H, DEPTq COSY, HSQC, HMBC). Differences among lethality percentage means were compared using the Tukey test ($p < 0.05$). Lethal concentrations (LC_{50} and LC_{90}) were determined with the PROBIT procedure.

Results

The methanol extract (ME) showed a yield percentage of *4.10%*, the ethyl acetate extract (EAE) 3.86%, and the hexane extract (HE) 1.09%. ME exhibited the highest yield, followed by EA and HE, respectively. The HE had the best LC_{50} (0.99 (0.86-1.10) mg/mL) compared to EA (2.56 (2.45-2.65) mg/mL), but no significative difference was found in LC_{90} compared with EAE (3.00 (2.74-3.35) mg/mL and 3.30 (3.26-3.56) mg/mL respectively). ME does not present a dose-response behavior therefore, LC_{50} and LC_{90} cannot be calculated. EAE extract was chosen to perform the bio-guided separation due to the highest yield percentage and anthelmintic activity. EAE was separated into 61 samples and grouped into three fractions (C1F1, C2F2 and C3F3) according to its chemical similarity. C1F1, C2F2 and C3F3 were evaluated against L_3 *H. Contortus* infective larvae where C1R2 was the most bioactive, followed by C1R1 and C2R3. Fraction C1R1 was selected to continue with de separation due to major yield percentage compared to C1R2. The separation of C1R1 was grouped into 9 fractions, with C2F7 showing the highest larvicidal activity and being selected to try to isolate a bioactive molecule. In Column 3, where C2F7 was separated, a colorless needle crystal in sample 13 (C313S) was observed. The larvicidal activity was determined and showed CL_{50} 0.13 (0.11 - 0.14) mg/mL) and CL_{90} 0.40 (0.37 - 0.44) mg/mL. Compared with EAE larvicidal activity, it is considerably more active. The structure of C313S was determined,

resulting in a new sesquiterpene that has not been previously reported, which we named cinic acid. It has a yield percentage of 0.01% regarding the extract.

Conclusions

The bioactive compound responsible for the larvicidal activity against *Haemonchus contortus* L_3 in the *Artemisia cina* ethyl acetate extract was identified as a new sesquiterpene, previously unreported, and named cinic acid.

Acknowledgments

This research was funded by the Support Program for Research and Technological Innovation Projects (PAPIIT-UNAM), titled: Evaluación del efecto tóxico del extracto *n*-hexánico de *Artemisia cina* y cinaguaiacina sobre los parámetros bioquímicos en sangre y alteraciones anatomopatológicas en ratas Wistar después de su administración por vía oral (IA204822). Arango-De la Pava was supported by Postdoctoral Grants Program from National Autonomous University of Mexico.

1548 - Anthelmintic efficacy comparison of endectocides, levamisole and albendazole in Tamuín, SLP. In naturally infected calves with gastrointestinal nematodes in a technified grazing system

Author: Jorge Alberto Carrillo Cortés, Dora Romero, Milagros González Hernández, Rigoberto Gutiérrez Molina, Francisco Martínez Ibañez, Meliton Lara Rocha, Corache García Pulido, Manuel Moctezuma García, Horacio Herrera Centeno, Alberto Ruiz San Martín, Mario Castro Rosas

Objectives
We describe the anthelmintic efficacy of four active ingredients: Ivermectin (IVM), Moxidectin (MOX), levamisole (LVS) and albendazole (ABZ), endectocides and anthelmintics with different mechanisms of action and degrees of residuality during five years of follow-up. Focusing on generating baseline data which is necessary to design strategic and efficient control programs according to the most effective molecules to control gastrointestinal nematodes (GIN).
Material and methods
Fecal sampling was carried out on four farms dedicated to calves fattening, once a year during 2018, 2019, 2021, 2022 and 2023 in Tamuín, SLP. The samples were processed by the Mc Master technique to detect positive calves with more than 300 eggs per gram of feces (HPG) on average and establish treatment groups using the oviposition reduction test (FERC). The treatment groups were as follows: (1) control or no treatment (NT), (2) IVM 0.2 mg/kg, (3) MOX 0.2 mg/kg, (4) LVS 3.75 mg/kg kg, and (5) ABZ 5.0 mg/kg. Treatment was applied to each group on day zero based on body weight and route indicated in each product. In all cases, follow-up was done by collecting fecal samples on day 14 post-treatment. The effectiveness of each treatment was calculated in the reduction of parasite load of treated cattle compared to NT group according to the World Association for the Development of Parasitology protocol. Treatments were considered effective if the geometric mean parasite load was reduced by ≥90% compared to the NT group. Larval pool culture was also carried out to determine the genera of existing nematodes.
Results
Calves in the ST group continued to be positive for GIN infections for the duration of the follow-ups. The overall efficacy percentages of the treatments were: IVM 17%, MOX 23%, lower than the expected 90%, LVS 90% and ABZ 95% efficacy, both treatments showing a significant statistical difference to the groups treated with endectocides and ST ($P > 0.05$). The genera found were *Cooperia* spp. (60%), *Haemonchus* spp. (30%) and *Oesophagostomum* spp. (10%).
Conclusions
The data show a first record in Mexico of resistance to both endectocides (IVM, MOX), established under these conditions; currently, treatments with ABZ and LVS providing an economically profitable treatment despite, these products have been available for years in the Mexican market and could be an alternative based on coproparasitoscopic diagnosis.

1551 - Comparative efficacy of single active macrocyclic lactone drenches with a novel dual active ML plus levamisole injectable drench assessed using faecal egg count reduction tests in Australian cattle.

Author: Sally Oswin, Frederico Siburg-Moreira

Objectives

To evaluate the performance of a new combination injectable dual active drench containing an ML (doramectin) and levamisole [Valcor™] in weaner cattle aged 3-12 months of age. This drench was evaluated against single active ML's where macrocyclic resistance was suspected or confirmed in twenty-one faecal egg count reduction tests (FECRT's) all around Australia.

Material and methods

Twenty-one properties were selected in regionally diverse locations around Australia, both beef and dairy breeds were represented. Enrolled properties had either a history of known ML resistance as identified by historic drench check results, or ML resistance was confirmed through a drench check conducted on a group of animals from the same cohort as those enrolled before the study was initiated. Weaners on each property had individual faecal samples collected on day -7 (+/-2 days) and were then allocated to one of three to five treatment groups of 15-20 animals according to a randomized complete block design. Animals were blocked on the basis of their faecal egg count (FEC), which was determined using the mini-FLOTAC method. All faecal samples collected on day -7 with ≥10 eggs per gram were pooled and cultured. On day 0 one group received the novel doramectin plus levamisole drench by injection and the other groups received a different single active ML formulation given by injection or pour-on or they remained untreated. Where a pour-on drench was used, that group of animals was moved to a separate paddock for the next 14 days of the study to avoid spreading the pour-on treatment to other groups through licking. On day 14, all animals had a faecal sample collected again for individual FEC, determined using the mini-FLOTAC, and the samples from each group were pooled by group prior to larval culture. Cultured larvae from faecal samples were identified to at least genus level and wherever possible, to species level. Percentage efficacy for each treatment group was calculated using the least squares (arithmetic) and geometric means.

Results

Over the 21 studies, the average geometric mean (GM) and arithmetic mean (AM) efficacy of each of the single ML drenches used was below 95%, while that of the doramectin and levamisole combination was 99.7% (GM) and 99.3% (AM) respectively. Resistance to single active ML drenches was identified in all of the following nematode genera: *Cooperia, Haemonchus, Ostertagia, Trichostrongylus* and *Oesophagostomum.* Of the twenty-one studies conducted, efficacy below 95% of the doramectin and levamisole combination was identified in only one study in *Haemonchus* (GM and AM) and *Trichostrongylus* (AM) nematodes.

Conclusions

This data indicates that ML resistance in young cattle in Australia is common and using a combination injectable drench containing both an ML (doramectin) and levamisole

was highly effective on farms where ML resistance was established. In addition, a significant difference in efficacy was also identified when a common ML active was given according to label directions via injection versus pour-on application with the former being more efficacious.

1577 - Resistance to commercial acaricides in Rhipicephalus microplus tick populations from Western Pará State, Brazil

Author: Poliana Leão Peleja, Ana Beatriz Barbosa Sousa, Daniela Bianchi, Raidel Reis Santos, Antonio Humberto Hamad Minervino

Objectives

We aimed to evaluate the efficacy of commercial acaricides containing amitraz, doramectin, and ivermectin against *Rhipicephalus microplus* populations from various cities in the Western Pará State.

Material and methods

We sampled engorged female ticks attached to cattle originated from four cities (Novo Progresso, Uruará, Rurópolis, and Mojuí-dos-Campos) located in the Lower Amazon and Southwest Pará mesoregions, Western Pará State. Ticks were morphologically identified as *R. microplus* and incubated (at 27–28 ºC and 80–90% humidity) for 14 days until oviposition. The resulting larvae were subjected to a Larval Packet Test using three chemical bases: ivermectin, doramectin, and amitraz. The solutions were prepared through serial concentrations of the acaricides (0.025%; 0.05%; 0.075%; 0.1%; 0.25%; 0.5%; 0.75%; and 1%) in a mixture containing two parts of trichloroethylene and one part of olive oil as diluent. Control packets contained only the diluent and each test was made in triplicate. Following a 24-hour exposure to the acaricides, both surviving and deceased larvae were tallied, and mortality rates were calculated. The drug mortality was calculated considering the larval mortality rate of the acaricide at a given concentration and the mortality rate of the control group using classical formula. A tick field sample was categorized as resistant if the mortality rate fell below 95%. The lethal concentration 50% (LC_{50}) was calculated using PROBIT analysis. A multivariate model assessed the relationship between mortality rates and various factors, including city, acaricide drug, concentration, and their interactions.

Results

At a standard concentration of 1%, the larval mortality rates were as follows: doramectin exhibited rates of 89.6%, 81.6%, and 91.1% for Uruará, Novo Progresso, and Rurópolis, respectively; ivermectin showed rates of 91.0%, 81.4%, and 95.1% for Novo Progresso, Uruará, and Mojuí-dos-Campos, respectively; and amitraz recorded rates of 79.5% and 80.9% for Novo Progresso and Mojuí-dos-Campos, respectively.

Overall, the acaricides proved ineffective against *R. microplus* populations from Mojuí-dos-Campos, Uruará, Rurópolis, and Novo Progresso, with the exception of ivermectin, which, at a 1% concentration, was effective against Mojuí-dos-Campos tick populations (95.1%).

The multivariate analysis indicated significant effects of city, acaricide type, and concentration on larval mortality rate ($P < 0.001$). Doramectin had the lowest mortality rate (34.2%) among the tested drugs, followed by amitraz (64.6%) and ivermectin (59.3%). The mortality for *R. microplus* larvae were higher in Uruará and Rurópolis compared to the other municipalities. No effects were observed in the mortality rate for the interactions Concentration*City ($P = 0.133$) and Concentration*Drug (0.247)

Regarding LC_{50}, drugs had a wide range according to different cities. For doramectin, we found 2,025 ppm (1,431 to 2,865 95% CI), 5,584 ppm (3,607 to 8,646 95% CI), and 2,885 ppm (2,010 to 4,074 95% CI) in Uruará, Novo Progresso, and Rurópolis, respectively. For

ivermectin LC_{50} was 514 ppm (312 - 844 95% CI), 699 ppm (437 to 1119 95% CI), and 2,715 ppm (1,810 to 4,074 95% CI) in Uruará, Mojuí-dos-Campos, and Novo Progresso tick populations, respectively. Amitraz had the most dissimilar LC_{50} with Mojuí-dos-Campos populations having more than 10 times higher concentration (7,483 ppm; 3,978 to 14,078 95% CI) when compared with Novo Progresso ticks (611 ppm; 466 to 802 95% CI).

Conclusions

Our findings indicated that commercial acaricides based on amitraz, ivermectin, and doramectin were mostly ineffective and showed variations in mortality rates among target populations for controlling *R. microplus* ticks in Western Pará. To our knowledge, this represents the first documentation of acaricide resistance involving macrocyclic lactones (doramectin and ivermectin) in Pará state, Amazon, Brazil.

1594 - Amazonian bioproducts as alternative for the control of the cattle tick Rhipicephalus microplus

Author: Ana Beatriz Barbosa Sousa, Poliana Leão Peleja, Daniela Bianchi, Antonio Humberto Hamad Minervino

Objectives

The present study aims to evaluate the potential of oils derived from two Amazonian plants, *Carapa guianensis* and *Copaifera reticulata*, in controlling the tick *R. microplus*.

Material and methods

Oils were obtained from two Amazonian plants, Andiroba (*C. guianensis*) and Copaíba (*C. reticulada*). Andiroba and Copaíba oils are widely used by traditional populations in the Amazon region for different purposes. Andiroba oil was extracted by seeds cold pressing while Copaíba oleoresin was collected directly from the trunk of an adult tree through drilling. Field samples of engorged female ticks were obtained from cattle raised in Pará State, Amazon. Ticks were morphologically identified as *R. microplus* and incubated (at 27–28 ºC and 80–90% humidity) until oviposition. The resulting larvae were used for Larval Immersion Test (LIT) to access the efficacy of natural products. Each oil it was tested in four different concentrations 10%, 5%, 2.5% and 1.25%. These solutions were prepared by dilution with Tween 80 at 1% concentration. For the LIT, approximately 300 larvae were immersed in each concentration of Copaíba and Andiroba oils and shaken for 5 minutes. After that, they were placed in filter paper, sealed, incubated for 24 hours, and the live and dead larvae were then tallied. Completely immobile larvae or larvae with only leg movements, without displacement, were considered dead. In each test, two controls were used, one using only distilled water and other using Tween 80. Five repetitions were performed for each LIT at each concentration. The acaricidal effect of each oil tested was calculated based on the mortality rate (%) caused in the larvae, corrected by the Abbot formula considering the mortality of the control group (Tween 80).

Results

In treatment with different concentrations of Andiroba oil, *R. microplus* mortality rates of 99.8%, 81.7%, 15.4%, and 6.3% were observed in treatments with 10%, 5%, 2.5 % and 1.25% concentrations, respectively, with mortality in the control groups 7.8% and 8.3% for larvae treated with distilled water and Tween 80, respectively. Regarding Copaíba oleoresin, the *R. microplus* larval mortality rate was 99.8%, 96.8%, 98.8% and 4.9% at concentrations of 10%, 5%, 2.5% and 1.25%, respectively. Controls showed mortality of 2.25% and 0.87% for larvae treated with distilled water and Tween 80, respectively.

Conclusions

Andiroba oil was effective for controlling populations of the *R. microplus* tick in Western Pará through LIT only at a concentration of 10%, while Copaíba oleoresin was effective at concentrations equal or above 2.5%. Both Andiroba and Copaiba show potential to be used for the formulation of a bioproduct from Amazon to control the cattle tick.

1596 - EVALUATION OF STRAINS OF Metarhizium anisopliae (s.l.) ISOLATED FROM LIVESTOCK SOILS ON THE MORTALITY AND REPRODUCTION OF Rhipicephalus microplus

Author: Francisco Velázquez Sarmiento, Roger Iván Rodríguez Vivas, Miguel Ángel Alonso Díaz, Dora Romero Salas, Agustín Fernández Salas

Objectives
The objective was to evaluate the acaricidal effects of strains of *Metarhizium anisopliae* sensu lato (s.l.) isolated from cattle farms against adult females, oviposition, and egg hatching of *Rhipicephalus microplus (Ixodida: Ixodidae*).
Material and methods
Soil samples were taken from 20 bovine production units in the municipality of Veracruz, Veracruz. *M. anisopliae* strains were isolated using the bait technique with greater wax moth larvae (*Galleria mellonella*). Fungi grown on *G. mellonella* were identified using taxonomic keys based on morphology, reproductive structures, spore shape and size, and growth characteristics. The isolated spores were reseeded on Sabouraud dextrose agar, bacteriological agar, yeast extract, and 250 mg of chloramphenicol and were incubated at 27±2 °C and 80-90% humidity. The MaV25 strain, evaluated in previous studies, served as a positive control. After 21 days of cultivation, conidia were collected, suspended in sterile distilled water containing 0.1% (v/v) Tween 80, and evaluated for acaricidal effects on *R. microplus*, including the percentage of mortality at 20 days through immersion tests on engorged females, and inhibition indices of oviposition (IOVIP) and egg hatching (IECLO) at a dose of 1×10^8 conidia/ml. The Kruskal-Wallis test was used to assess the effects of strains on IOVIP and IECLO, and survival analysis for mortality curves was conducted using SPSS V26.
Results
All *M. anisopliae* (s.l.) strains achieved 100% mortality in engorged females, with a significant effect on IOVIP (16.45 - 56.38%) and moderate impact on IECLO (5.24 - 32.68%). Strains MaV76 and MaV25 showed a greater impact on the mortality curve (73% on day 8, $p<0.05$). MaV72, MaV76, and MaV25 were more effective ($p<0.05$) for IOVIP (42.71%, 56.38%, 48.01%) and IECLO (27%, 32.68%, 32.4%).
Conclusions
The isolates of *M. anisopliae* from livestock soils in this study proved to be highly effective in inducing mortality in engorged adult females of *R. microplus*. They exhibited a significant impact on oviposition and demonstrated moderate effectiveness in reducing egg hatching

1598 - EXTERNAL AND INTERNAL STRUCTURAL DAMAGES IN Rhipicephalus microplus CAUSED BY STRAINS OF Metarhizium anisopliae (s.l.) ISOLATED FROM LIVESTOCK SOILS.

Author: Francisco Velázquez Sarmiento, Roger Iván Rodríguez Vivas, Miguel Ángel Alonso Díaz, Justino Figueroa Velarde, Agustín Fernández Salas, Dora Romero Salas

Objectives
The aim was to histopathologically determine external and internal structural damage (cuticle, salivary glands, intestine, Malpighian tubules, and ovaries) caused by *M. anisopliae* to *R. microplus* ticks.
Material and methods
The immersion test of engorged adult females of *R. microplus* ticks was conducted with 14 strains of *M. anisopliae* isolated from livestock soils, using a dose of 1×10^8 conidia/ml. The ticks were observed for 20 days, and those identified as dead were preserved in 10% buffered formalin. Based on the results obtained in terms of percentage and speed of mortality, inhibition of oviposition, and hatching of eggs, strains MaV76, MaV71, MaV80 (high, medium, and low efficiency, respectively), and MaV25 (positive control) were classified and selected. Dead ticks from days 4, 6, 8, and 10 were processed for histopathology, they were dehydrated in increasing concentrations of ethyl alcohol, cleared in xylene, embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin-eosin. Slides were examined using a Leica® DM 500 microscope with a digital camera. The observed lesions were classified as grade 1 (moderate), grade 2 (intense), and grade 3 (severe) based on changes in the nucleus, cytoplasm, basal membrane, and cellular architecture.
Results
The salivary glands and the intestine were the most severely affected. In the intestine, the architecture of the membranes of germinal, residual, and sessile digestive cells was disrupted, with changes in the number and shape of digestive granules and vacuoles. Salivary glands and Malpighian tubules exhibited nuclei with fragmented chromatin, up to "nuclear ghosts," and cytoplasmic membranes formed a homogeneously eosinophilic mass. Oocytes of different stages underwent a degenerative fibrillar to coagulative process, losing the chorion, vitelline granules, and, in some cases, the germinal vesicle. The cuticle showed significant damage to epidermal cells. Strains MaV76 and MaV25 caused the most damage to various organs on different evaluation days.
Conclusions
The isolates of *M. anisopliae* from livestock soils in this study caused external and internal structural damage in *R. microplus*. The intensity of these lesions tends to correlate with the level of efficiency demonstrated in terms of mortality rate, inhibition of oviposition, and hatching.

1611 - Frequency of Cryptosporidium spp. in lactating calves from cattle ranches in the temperate zone of Veracruz, Mexico.

Author: Dora Romero Salas, Anabel Cruz Romero, Milagros González Hernández, Marco Antonio Alarcón Zapata, Melina Maribel Ojeda Chi, R Rodríguez Molina, Jorge Alberto Carrillo Cortes

Objectives

Cryptosporidium spp is a protozoan that causes diarrhea associated with dehydration, colic, and diverse etiologies complications in neonatal calves. Therefore, our study aimed to determine *Cryptosporidium* spp frequency in lactating calves from one to 60 days of age in cattle ranches in the temperate zone of Veracruz, Mexico.

Material and methods

A cross-sectional epidemiological study with a convenience sampling was carried out with lactating calves from cattle ranches located in four municipalities (Acatlán, Landero y Coss, Miahuatlán and Naolinco) in the temperate zone of Veracruz, Mexico. The sample size was estimated with Win Episcope version 2.0, in the modality of estimating percentages, with a prevalence of 50%, a margin of error of 10% and a confidence level of 95%, which gave a n=300 animals. The animals included in this study were lactating cattle from one to 60 days of age, regardless of sex, presence or absence of diarrhea. Sampling was conducted from March to June 2023. A direct stool sample was taken from the rectum with a properly identified latex glove. Samples were transported under refrigeration (4°C) to the Parasitology laboratory of the Diagnostic Unit at the "Torreon del Molino" ranch of the Faculty of Veterinary Medicine and Zootechnics of the Universidad Veracruzana. They were first analyzed by the modified Faust centrifugation method (Leventhal and Cheadle, 1992), then stained by the modified Ziehl-Neelsen technique (Casemore et al., 1985). A general and an individual survey were applied in each cattle ranch. Data were captured in an Excel® Microsoft spreadsheet for each of the variables and descriptive statistics were performed. Frequency was determined with the statistical software STATA version 14.0.

Results

The overall frequency of *Cryptosporidium* spp was 66.6% (CI95% 60.9-71.9), the highest frequency was found in the calves sampled in the ranches of the municipality of Miahuatlan with 82.6% (CI95% 71.8-90.1) and the lowest in the calves of Landero y Coss with 52.0% (CI95% 40.2-63.5); however, positive animals were found in all the sampled ranches of the four municipalities. Respect the age of the animals, the highest frequency of *Cryptosporidium* spp. was found in calves 1-15 days old with 73.7% (CI95% 64.8-81.1) and the lowest frequency in calves 40-60 days old with 44.4% (CI95% 26.0-64.3). According to the sex of the animals, the highest frequency of *Cryptosporidium* spp. occurred in females with 69.3% (CI95% 61.5-76.2). The frequency in relation to the consistency of semi-liquid and liquid feces was 75.6% (CI95% 66.5-82.9) and 72.3% (CI95% 66.5-82.9) respectively, which proves that the main sign of *Cryptosporidium* spp. in lactating calves is the presence of diarrhea, which can sometimes be fetid.

Conclusions

The presence of *Cryptosporidium* spp oocysts was identified in lactating calves on cattle ranches in four municipalities located in the temperate zone of Veracruz, Mexico. The

highest frequency of *Cryptosporidium* spp occurred in lactating calves 1-15 days of age, in females and in those with liquid stool consistency.

1653 - Failure in the control of flukes (Fasciola hepatica and paramphistomids) using mixtures of anthelmintics in cattle in Chiapas, Mexico

Author: Roberto González Garduño

Objectives

The objective of this study was to know the effectiveness of mixed anthelmintics on trematodes in Chiapas, Mexico.

Material and methods

The Effectiveness of mixed Triclabendazole + Albendazole + Ivermectin was determined in two farms (n1= 80 and n2=30), in one farm was used Ivermectin + Closantel (n=21) and Ivermectin + Clorsulon (n=21). The study was carried out from October to December 2023. The area is warm and humid with flat sites with clayey soils, generally flood-prone with high prevalence of flukes (32-41%). Rumen and liver fluke (*Fasciola hepatica*) eggs were differentiated using methylene blue in sedimentation technique. At least three visits were made to each farm. The first to determine the fecal egg count (FEC) of flukes, the second to deworm the cattle and the third to determine the effectiveness of the anthelmintic (AH). At least 10 animals remained without deworming as positive controls. Effectiveness was calculated using the fecal egg count reduction test (FECRT), respect to control = 100 × (*1-T2/C2*), where T2 represent the FEC after the treatment, while C2 represents the FEC of the control group (without deworming) after to deworming.

Results

Trematodes (liver and rumen fluke) represents an economic problem in livestock as they affect animal health and productivity. The combination of Ivermectin + Closantel showed 46% effectiveness in *F. hepatica* and 66.4% in rumen fluke, while Ivermectin + Clorsulon had 89.8% effectiveness in *F. hepatica* and 51.9% in rumen fluke, while Triclabendazole + Albendazole + Ivermectin was 45% effective in *F. hepatica* and 39% in rumen fluke.

Conclusions

With these results, it is concluded that the use of anthelmintics to control trematodes indicates anthelmintic resistance, so it is necessary to search for other alternative solutions and consider a comprehensive control to reduce the effect of trematodes on cattle production.

1655 - Prevalence of ticks and thick transmitted haemoparasites of beef cattle in the north region of Puebla, México.

Author: Miguel Angel Fernando-Morales, Samuel Luis-Rojas, José Victoriano Ramírez-Romualdo, Luis Manuel Hernández Loaiza, Cipatli García-Dalmán

Objectives

Babesia spp. and *Anaplasma spp.* are haemoparasites that cause direct losses attributed to acute illness and death, premature slaughter and rejection of some parts during meat inspection, are considered some of the major impediments to the health and productive performance of cattle. Babesiosis and anaplasmosis are the most important tick-borne diseases in beef cattle. In the northern region of Puebla, México there are ideal environmental conditions (temperature and humidity) for the presence of ticks. Investigation on haemoparasites and their transmitters in this area of Mexico is not available. The aim of present study was to determine the prevalence of ticks and thick transmitted haemoparasites of beef cattle in the north region of Puebla, México.

Material and methods

In the present work, ticks of each stage and blood samples were collected on cattle from six livestock farms located in Tepango de Rodríguez, Ahuacatlán and Coatepec communities in the north region of Puebla, México. The average temperature range is 10 to 28°C, and annual rainfall range from 1500 – 2000 mm. A total of 139 blood and ticks samples were collected from July to November of 2023. The basic data of management were recorded, including the number of animals on the farm, body condition score, deworming program and veterinary service. The tick species were identified by examining their morphological characteristics and Giemsa stained thin blood smears were examined for haemoparasites.

Results

A total of 3,635 specimens *Rhipicephalus (Boophilus) microplus* (100% of total) were collected. The most infested animal body areas were anogenital, udder and abdomen. An overall prevalence of 9 % haemoparasites was recorded for all samples examined, *B.bigemina* and *A.marginale* were 2 % and 7 % respectively, one sample had both haemoparasites.

Conclusions

These results confirm the existence of ticks as an ectoparasite of cattle, vector of haemoparasites, in the north region of Puebla, México. The prevalence of tick infections was common and the haemoparasite infection are minor. The data obtained from this study would be useful for future effective parasitic disease prevention among cattle.

1657 - Excretion dynamics of fecal egg counts of gastrointestinal nematodes in lactating calves in Chiapas, Mexico

Author: Claudia Yesenia León González, Roberto González Garduño

Objectives
For decades, anthelmintics have represented the main way to control gastrointestinal nematodes (GIN), however, their excessive use has led to the generation of parasites resistant to them, so their effectiveness has decreased considerably. The natural resistance of the animals is a possibility, so the hypothesis proposed is that immunity experience a progressive development against GIN that increase in age, thus reducing parasitic infections and therefore requiring fewer applications of anthelmintics. To test this hypothesis, an experiment was carried out with the main objective of knowing the population dynamics of GIN in calves from birth to eight months of age.
Material and methods
The study was carried out in the municipality of Salto de Agua, Chiapas, Mexico. Using sedimentation (McMaster) and flotation techniques. The number of GIN eggs from suckling calves was determined; one group was dewormed (n=14) and another group was left without deworming (n=14). Fecal samples were obtained every 21 days from the 28 calves for a period of six months and a total of 9 samplings were carried out. The samples were processed using McMaster and sedimentation techniques to know the fecal egg count (FEC) dynamics. In each sampling, the samples with the highest number of parasites were selected for copro-cultures to determine the main parasite genera.
Results
The fecal egg counts showed average values of 285 ± 396 eggs per gram of feces (epg) of trichostrongylids, of which the *Cooperia* species was the most prevalent (80%), and in a low percentage *Haemonchus* and *Trichostrongylus*, while *Strongyloides* was abundant especially during the rainy season (August-October), and *Oesophagostomum* was observed in a very low proportion (<5%). Up to eight months of age, the calves showed similar epg and no reduction was observed due to age. The anthelmintic (Triclabendazole + Albendazole + Ivermectin) used to control GIN was not effective, showing less than 85% effectiveness.
Conclusions
In conclusion, no significant population decrease in gastrointestinal nematodes was observed as the calves increased in age, nor were there significant differences between the dewormed and non-dewormed groups.

1661 - Morphological diagnosis of gastrointestinal parasites in sheep slaughtered in Tabasco, Mexico

Author: Jonathan Cárdenas Guzmán, Roberto González Garduño

Objectives

The identification of the species of gastrointestinal parasites is important because the control measures taken on farms depend on the prevalent species, so the objective of this work was to know the prevalence of the main species of gastrointestinal parasites in sheep slaughtered through morphological diagnosis in Tabasco, Mexico.

Material and methods

The sheep samples were collected in a particular slaughterhouse located in the Jolochero, municipality of Centro, Tabasco. The sheep were entered into the slaughter area where data on place of origin, age and sex were taken. Fecal extraction was also performed. Subsequently, the animals were sacrificed in accordance with NOM-033-SAG/ZOO, 2014. After slaughter, the gastrointestinal contents were recovered. To obtain the parasites from the small intestine, the entire organ was removed, and the contents were collected in 350 ml containers. Similarly, to recover the abomasum parasites, an incision was made in the organ, and then emptied into a container. The samples obtained were transported to the animal parasitology laboratory where the fecal egg count (FEC) of nematodes was carried out by flotation with the McMaster technique in order to obtain the number of eggs per gram of feces (epg). The contents of the abomasum were washed with running water and the adult gastrointestinal nematodes were counted and identified.

Results

As part of the results, identification was carried out based on the corresponding measurements of each species and a prevalence of gastrointestinal parasites has been found in sheep of 8% *Strongyloides papillosus*, 8% *Fasciola hepatica*, 3% Paramphistomids, 37% *Haemonchus contortus*, 22% *Cooperia curticei*, 30% *Trichostrongylus colubriformis* and 8% *Moniezia expansa*.

Conclusions

It is concluded that there was a high presence of gastrointestinal parasites in sheep, so it is necessary to adopt management measures to reduce the prevalence of parasites in the flock.

1663 - Use of essential oils for the control of Riphicephalus microplus nymphs of cattle in the Tropics of Mexico

Author: Juan Manuel Hernández Domínguez, Roberto González Garduño

Objectives

The main ectoparasites include mites, ticks, fleas, lice, and flies, which affect the health of animals and reduce productive levels because many of them are hematophagous, which implies that livestock is subjected to constant stress due to high number of parasites present in the environment. Furthermore, the economic importance of these parasites increases due to the constant use of ixodicides, acaricides or insecticides that have led to the obtaining of specimens resistant to the main products used. The hypothesis proposed is that the excessive use of these chemicals has generated resistance in ticks, so more environmentally friendly control methods such as essential oils should be sought, so the objective of the experiment was to determine the resistance of ticks towards the main ixodicides and determine the lethality rate with the application of essential oils extracted from aromatic plants.

Material and methods

A collection of adult ticks was carried out and placed in perforated Petri dishes to keep them at 25 °C and wait for oviposition. Ten days after hatching, the first instar nymphs were used to determine the mortality rate when applying the immersion method. An organophosphate (dichlorvos) was evaluated in different doses and the lethality rate was determined. Part of the nymph population was also tested using essential oils, specifically oregano, cinnamon, cumin, lemongrass and pepper. Previously, the distillation of these products was carried out using Clevenger equipment and the oil was kept refrigerated at 4 °C.

Results

The dose recommended by the manufacturer (1.14 µg/mL) did not cause mortality of the nymphs, so resistance to dichlorvos is presumed. For its part, the preliminary results of the use of essential oils indicate that at very high doses the total mortality of the nymphs occurs (20 mg/mL), while the lethal dose 50 is found in values less than 2 mg/mL for cumin oil and which requires confirmation because doses lower than this value are not used, for its part, lemon grass and pepper oil were the ones that had the highest doses since with 10 mg/mL a survival rate of 35%.

Conclusions

The lethal dose of a conventional control applied was 10 times higher than that recommended by the brand and in the case of essential oils, the majority presented good results with the applied doses. Oils represent the possibility of moving towards an unconventional and more environmentally friendly system with the aim of interspersing control methods to reduce the resistance present in ticks.

1684 - Evaluation of ocular and vulvar FAMACHA to identify cattle with high loads of gastrointestinal parasites

Author: Mario Antonio Mendoza Nuñez, Heladio Moreno Melo, Alfredo Meza Gregorio, Francisco Mayoral Vargas, Citlali Estefania Sollano Mendieta, Maria Benedicta Bottini Luzardo

Objectives

To evaluate the application of the FAMACHA© ocular and vulvar technique for the identification of cattle with high loads of gastrointestinal parasites.

Material and methods

The present research was carried out at the UPP (Los Lagartos) located in the town of Cuiji, municipality of Cuajinicuilapa, Guerrero, Mexico. 44 cattle were sampled (61.4% females, 38.6% were males, 75% were red and white Brahman and only 25% were Swiss-Bu crosses). At the time of evaluation, the animals had been stabled for a month, they had previously been grazed with no history of deworming, the color of the ocular and vulvar mucosa was determined using the FAMACHA© method, in addition a feces sample was obtained to determine the parasitic load using the Mc-Master method (Sixto, 2011).

Statistical analysis was performed in the IBM SPSS Statstics 25 program. The statistical association between parasite load and ocular FAMACHA, vulvar FAMACHA, was performed using a Pearson Chi-Square test.

Results

The overall prevalence was 93.2%. 77.3% were positive for coccidia, 50% for nematodes and 4.5% for the cestode Moniezia sp. Only 14% of animals had high nematode loads (≥500 H.P.G.), and only 16% had high coccidia loads (≥500 O.P.G.).

40.9% of the animals had ocular FAMACHA© of 2 and 59.1% had ocular FAMACHA© of 3. That is to say, the vast majority had good coloration of the mucous membranes. The technical recommendations (in small ruminants) indicate that deworming in these categories is not necessary.

The Chi-Square test did not show a statistically significant association between the shade of the ocular mucosa and the parasitic load of nematodes (X^2: 1.907, gl: 1, *P*: 0.167) or coccidia (X^2: 0.013, gl: 1, *P*: 0.909) similar to that reported by Lazo and Gonzáles (2020).

In the case of vulvar FAMACHA©, 29.6%, 63% and 7.4% of the females had FAMACHA© of 2, 3 and 4 respectively. For statistical analysis, categories 3 and 4 were grouped as category 3.

The Chi-Square test did not show a statistically significant association between the shade of the vulvar mucosa and the parasite load of nematodes (X^2: 0.934, gl: 1, *P*: 0.33) or coccidia (X^2: 0.909, gl: 1, *P*: 0.34).

Conclusions

Several authors have associated pale ocular mucous membranes with high loads nematode parasites in grazing sheep (Ensuncho et al., 2014 and Arece and López et al., 2013). In relation to the application of FAMACHA© in cattle, there are few published studies. In our study no we found a statistically significant association between the parasite load of nematodes or coccidia and the tone in the ocular o vulvar mucosal determined by ocular FAMACHA© and vulvar FAMACHA©.

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1715 - Finding of sarcocyst in Holstein´s cull cow meat. A report case

Author: Maria de los Angeles Robles Mota, Maria Fernanda Anguiano Pérez, Manuel Andres González Toimil, Jorge Alfredo Cuéllar Ordaz, Crisoforo Mercado Márquez, Cesar Cuenca Verde, Rosa Isabel Higuera Piedrahita

Objectives

To describe the sarcocystis founded in histological slides of Holstein cull cow meat

Material and methods

The Holstein cull cows were 5 to 7 years old animals that came from the dairy basin region in Mexico. The animals were slaughtered and 48 hours later we obtain a sample of a rib eye cut of every animal.

Fifty meat samples about 2g were taken of *longissimus dorsi* muscle after 72 hours slaughtering. The samples were fixed in formaldehyde at 10% per 48 hours and dehydrated with increasing alcohol solutions and xylene, then infiltrated and included in paraffin. The paraffin cubes were cutted from 8µm, the histological slides were colored with hematoxilin- eosin stain and the evaluation was made with an optical microscope with 10x, 40x and 100x objectives

Results

Sarcocystis spp. is an aplicomplexa intracellular parasite that affect herbivore animals as intermediate hosts, and humans too. Human can be infected by the consumption of microcysts in raw or under cooked meat, there are reports of cases in non-immunocompetent human patients. In Mexico the prevalence of sarcocystis has not been established because it does not cause serious damage.

The visual evaluation of rib eye cuts did not show any macroscopic cyst. Twenty samples presented microcysts inside the myocyte´s sarcoplasma that measured in average 15*30 µm. The microcysts had an oval and elongated shapes with thin wall, that can be appreciated basophil with hematoxilin-eosin stain, without eosinophilic or cellular infiltration. Some samples presented up to three cysts.

Conclusions

Sarcocystis were detected in histology evaluation in twenty samples of Holstein´s cull cow meat as a finding even though there were not macrocyst.

1723 - DETERMINATION OF THE EFFECTIVE DOSE OF INJECTABLE FOSFATRICLABEN PRODRUG IN CATTLE ARTIFICIALLY INFECTED WITH Fasciola hepatica

Author: FROYLAN IBARRA VELARDE, Miguel Flores-Ramos, Irene Cruz-Mendoza, Tania Rojas-Campos, Yolanda Vera-Montenegro, Alicia Hernández-Campos, David Tovar-Escobar, Rafael Castillo, Gerardo Francisco-Márquez, Pedro Ochoa-Galvan, Pablo A. Rojas-Reyes.

Objectives

The objective was to determine the effective dose of fosfatriclaben in experimentally infected steers.

Material and methods

On day 0 (zero), twenty fluke-free Holstein Friesian steers were orally infected with 500 *Fasciola hepatica* metacercariae. Ten weeks after infection, when the steers were confirmed to be positive for fluke eggs, they were ranked according to their fecal egg count, from highest to lowest number of eggs. Then, they were divided into 5 groups of 4 animals each, for the treatment. Group 1 (G1) served as an untreated control. G2, G3, and G4 were treated with a single dose of 4, 6, or 8 mg/kg/IM of fosfatriclaben prodrug, respectively. G5 received a commercial formulation of triclabendazole/ivermectin at 12 mg/kg/IM. Fecal analyzes were performed on days − 8, 0, 70, 75 and 105 to count the number of *F. hepatica* eggs, as well as count the adult stages of all experimental groups. On day 105, the steers were humanely euthanized to remove the flukes from the bile ducts and count them to assess the percentage of efficacy.

Results

The results exerted a fluke-egg reduction of 87.5, 99.7, 100 and 99.7%, and liver fluke reduction of 98.9, 100, 100 and 99.5%, for groups G2, G3, G4 and G5, respectively. The data obtained on the fluke length from the control group showed that the greater the number of flukes collected from a single animal, the smaller the average size of the flukes recorded.

Conclusions

It is concluded that the effective dose of injectable fosfatriclaben selected for bovines was 6 mg/kg/IM. Study financially supported by the project PAPIIT-IT200422, DGAPA-UNAM.

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1752 - Experimental vaccination of cattle using a recombinant peptide RmS-17 against Rhipicephalus microplus tick infestation under field conditions

Author: Rodolfo Lagunes Quintanilla, Roberto Castañeda Arriola, Rene Camas Pereyra, Edgar Castro Saines, Ruben Hernández Ortiz, Jaime Rangel Quintos

Objectives

The cattle tick *Rhipicephalus microplus* is considered one of the main concerns in cattle production in tropical and subtropical regions. Anti-tick vaccines may form an alternative tick control method to acaricides, and tick salivary proteins, such as Serpins, may be valuable as target antigens for developing anti-tick vaccines. In this study, we produced a recombinant peptide derived from Serpin RmS-17 protein using the *Escherichia coli* expression system. We characterized the efficacy for controlling *R. microplus* infestation under field conditions.

Material and methods

The study was conducted at the Experimental Site Pichucalco / National Center of Disciplinary Research in Animal Health and Safety of the National Institute for Forestry, Agricultural and Livestock Research (INIFAP) in Pichucalco, Chiapas, Mexico. Fifteen female calves were assigned to three experimental groups and immunized with three subcutaneous doses of the peptide RmS-17, Bm86, and adjuvant/saline alone. All animals were immunized in the neck using a 5 mL syringe and an 18 G needle on days 0, 30, and 50. The animals were checked regularly for signs of local reaction or clinical abnormalities post-immunization. Blood samples were collected from all 15 animals every week for 133 days. Samples were collected from the caudal vein into sterile tubes with no anticoagulant immediately before each immunization and maintained at 4 ◦C until further analysis by ELISA. To evaluate the effect of the experimental groups on the reproductive performance, adult female ticks (4.5–8 mm) were collected weekly. All the collected adult female ticks were weighed and kept individually in tick chambers to assess for oviposition and egg fertility. The efficacy of vaccine formulations was calculated by using standardized formulae. Finally, data were statistically analyzed with the software StatGraphics®.

Results

The immunization of cattle with the peptide RmS-17 and Bm86 antigen resulted in recovery of 40% and 51% fewer fully engorged adult female ticks compared to the control ($P < 0.05$), respectively; several ticks collected from both experimental groups were visibly damaged and showed effects such as physical damage, dry appearance, a dark-red coloration, and in some cases, death observed. There were no significant differences in tick weight. However, the oviposition and egg fertility were moderately reduced. The overall efficacy of the peptide RmS-17 was close to 70% against *R. microplus* infestations. Vaccination with the peptide RmS-17 and Bm86 resulted in significantly different IgG levels compared to controls from day 15 until the end of the experiment ($P < 0.05$). IgG levels in the peptide RmS-17-vaccinated calves peaked on day 30 after the second vaccination and achieved at least 3.20 OD units compared to the adjuvant/saline control. In contrast, Bm86-vaccinated calves increased after successive immunizations and peaked on day 42, and the IgG levels were 1.58 OD units at most. These results showed that all animals tested responded to the vaccines and were protected against tick infestations.

Conclusions

This study reports the first vaccination trial using a peptide RmS-17 derived from a Mexican *R. microplus* strain against natural tick infestations. The results allow us to conclude that the peptide RmS-17 can induce a specific immune response in cattle and confer protection against *R. microplus* in a Mexican tropical region. These findings highlight the importance of considering an integrated management approach for tick control and supporting further experiments with this antigen to demonstrate its effectiveness as a regional vaccine.

1753 - Protective efficacy of the peptide Subolesin antigen against Rhipicephalus microplus tick infestation under natural conditions

Author: Rodolfo Lagunes Quintanilla, Nancy Mendoza Martínez, Miguel Ángel Alonso Díaz, Octavio Merino Charrez, Agustín Fernández Salas

Objectives

The cattle tick *Rhipicephalus microplus* affects animal health, welfare, and production in tropical and subtropical zones. Anti-tick vaccines have been an effective alternative for cattle tick control instead of traditional chemical products. To date, the Subolesin antigen has shown efficacy for controlling tick infestation in cattle, and previous studies have shown that one peptide derived from this protein has been demonstrated to elicit a strong and specific humoral immune response. Based on these findings, we characterized the efficacy of the peptide Subolesin for controlling *R. microplus* tick infestation under natural conditions.

Material and methods

This study was conducted at the Centre for Teaching, Research, and Extension in Tropical Livestock (CEIEGT) from the Faculty of Veterinary Medicine of the National Autonomous University of Mexico (FMVZ-UNAM), Martínez de la Torre, Veracruz, Mexico. Twenty-four 12–15 month-old female calves were used in this study. Cattle were randomly assigned to four experimental groups of six animals each, the peptide Subolesin (group A), Bm86 (group B), both antigens in a dual vaccine (group C), and control (group D). Each calf was immunized with 3 doses of purified recombinant proteins formulated previously. Group C was injected at the left and right sides of the neck, and Group D received phosphate-buffered saline (PBS) and adjuvant. All animals were subcutaneously immunized on days 0, 30, and 65. Blood samples were collected from all 24 animals every 15 days for 170 days. Samples were collected from the caudal vein before each immunization and centrifugated to collect the serum for further analysis by ELISA and Western blot. To evaluate the effect of the experimental groups on the reproductive performance, adult female ticks (4.5–8 mm) were collected weekly. All the collected adult female ticks were weighed and kept individually in tick chambers to assess for oviposition and egg fertility. The efficacy of vaccine formulations was calculated by using standardized formulae. Finally, data were statistically analyzed with the software StatGraphics®.

Results

The effect of the peptide Subolesin was significant for adult female tick, oviposition, and egg fertility parameters ($P < 0.05$). The dual vaccine group showed significant differences in oviposition and egg fertility. In contrast, cattle immunized with Bm86 only have significant differences in oviposition ($P < 0.05$). These results showed 67% vaccine efficacy for the peptide Subolesin and 56% and 49% for the Bm86 and the dual vaccine, respectively. Antibody levels increased in cattle immunized with the peptide Subolesin, Bm86, and the dual vaccine but not in controls after each immunization and were significantly different ($P < 0.05$) until day 114. These results showed that all of the animals tested responded to the vaccine. Group A developed a strong, specific humoral immune response with high anti-pSubolesin IgG levels. The antibody levels in cattle immunized with the dual vaccine showed a moderate increase and decreased considerably at the end of the experiment. In contrast, a lower increase was recognized

in cattle vaccinated with Bm86 (±1OD). Western blot analysis showed an increased antibody recognition level of immunized bovine serum (collected at day 72) to the two antigens compared to the adjuvant/saline control. The anti-pSubolesin IgG produced from cattle recognizes tick proteins derived from *R. microplus* partially engorged female guts and salivary glands; additionally, antibodies against Bm86 recognized guts and ovaries. A band at the molecular weight of ~17 kDa is consistent with the peptide Subolesin from the *R. microplus* ticks, while the anti-Bm86 recognized a ~72 kDa protein.

Conclusions

The results of this study allow us to conclude that the peptide Subolesin can induce a specific immune response in cattle under field conditions. These results confirmed the efficacy of the peptide Subolesin to control *R. microplus* infestations, resulting in the reduction of tick populations in subsequent generations. However, further studies are needed to investigate an optimal vaccine design and formulation to find an accurate combination of antigens and their potential synergistic activity to demonstrate its effectiveness as a vaccine. In this work, we cannot rule out the possibility that the nature of the antigens, the concentration, and the adjuvants used have influenced the immune response from the bovines vaccinated with the dual vaccine. Therefore, additional trials are required before an anti-tick vaccine is based on two or more antigens.

1781 - Seroprevalence of Neospora caninum in a dairy cattle farm in the Northern region of Continental Portugal

Author: José Pedro Quaresma Silva, Paulo Capêlo, Ricardo Cabeças, Gonçalo Frouco, Sérgio Sousa

Objectives

Neospora caninum is an obligatory intracellular protozoan parasite that causes reproductive and productive losses, being responsible for significant economic losses in cattle. This study was conducted to investigate the seroprevalence of *N. caninum* and its influence on reproductive and productive parameters in a dairy cattle farm in the northern region of mainland Portugal. One hundred serum samples were analyzed using enzyme-linked immunosorbent assay (ELISA) tests for *N. caninum*. The seroprevalence in the farm was 28% (28/100, 95% CI: 19.70 – 38.01). A significant difference was observed in 'production' and 'gestation length' variables between seropositive and seronegative animals (P-*value*=0.007 and P-*value*=0.01), respectively. Regarding the 'abortions' variable, a correlation of P-*value*=0.07 and *odd ratio* (OR) of 3.77 (95% CI) were found. It can also be concluded that there are differences in the number of artificial inseminations (IA) and the calving interval, with seropositive animals requiring 1.56 times more IA for successful pregnancy. The mean calving interval (IEP) was 415 days in positive animals and 382 days in negative animals. Therefore, it was concluded that the *N. caninum* parasites has a negative impact on the productive and reproductive parameters of dairy cattle farms.

Keywords: *Neospora caninum*; seroprevalence; dairy cattle; productive and reproductive parameters; Portugal.

Material and methods

A total of 100 blood samples were collected on a dairy cattle farm located in Barcelos in November 2022. The municipality of Barcelos (41° 32' 05" N 8° 36' 54" W) is situated in the Northern region of mainland Portugal. It has a mild climate, characterized by an average temperature of 23ºC in the summer and 16ºC in the winter. Regarding precipitation, December experiences the highest rainfall (136mm), while July is the driest month with 13mm of precipitation, according to Weather Spark (2023). The farm is family-run with robotic milking and approximately 60 animals in production, housed in a free-stall system. Feed is provided in the manger through a total mixed ration (TMR) system, consisting of a corn silage, straw and concentrated feed. The silos are located outside the farm premises. The animals are vaccinated against Bovine Viral Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR) and *Clostridium* spp. Reproductive control measures include regular visits from the attending veterinarian every two weeks and the use of artificial insemination (AI) as reproductive management. The samples were collected in November 2022. Approximately 5 mL of blood was collected through coccygeal venipuncture into tubes without anticoagulant. The samples were labeled and stored at a temperature of -20ºC until analysis. All procedures were performed by trained professionals belonging to a reference laboratory in the region. The presence of anti-*N. caninum* IgG antibodies were detected using a commercial indirect ELISA kit (Civtest™ Bovis Neospora, Laboratories Hipra, Spain), following the manufacturer´s instructions. This kit is known for its high sensitivity and specificity (Alvarez-García et al., 2013). The optical density (OD) of each sample was measured at 405 nm using a

Microtiter Plate. For the test to be considered valid, the OD of the positive control had to be >0.9, and the ratio (OD positive control/OD negative control) had to be >0.5. According to the manufacturer, animals with S/P%≤ 10% were considered negative, while S/P%> 11% were considered positive. Data regarding productive and reproductive parameters were collected from the farm´s management assistance program for all cows, heifers and calves included in the *N. caninum* research. In this study, only animals that had calved at least once were included to evaluate their respective reproductive parameters. Data on age, number lactations, number of artificial inseminations, calving interval, number of abortions, gestational age at abortion, and milk production were collected from cows that met these criteria. Wilcoxon rank sum and Fisher's Exact tests were used to analyze the variables (production, gestation time and abortions) associated with *N. caninum* infection. To assess the relationship between *N. caninum* seropositivity and productive and reproductive variables (production, gestation time and abortions) in the study farm, the *R Core Team* (2022*) program* (*R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. URL https://www.R-project.org/) was used. A P-value<0.05 was considered statistically significant. The results will be presented as odds ratios (OR) with a 95% confidence interval (95% CI).

Results

The seroprevalence of *N. caninum* in the farm was 28% (CI: 19.70 – 38.01). A higher seroprevalence was observed in animals between two and three years old (71%, 20/28), compared to animals up to one year old (11%, 3/28) and animals over three years old (18%, 5/28). The number of lactations, the calving interval, and the number of inseminations per calving were some of the productive and reproductive parameters addressed in this study. The average number of lactations on the farm was 1.87, while in seropositive animals, the average number of lactations was 1.28, and in seronegative animals, it was 2.11. Regarding the average calving interval on the farm, we observed 391 days. In seropositive animals for N. caninum, the calving interval was 415 days, while in seronegative animals, it was 386 days. Finally, the average number of artificial insemination (AI) per calving in the study farm was 1.35. Seropositive animals had a higher number of AI per calving, around 2 AI's, while seronegative animals had an average of 1.28 AI per calving. Statistical analysis of the data showed a significant difference in the 'production' variable between seropositive and seronegative animals (W=736; P-value=0.007). This demonstrates that a statistically significant difference in production was observed between the studied groups. It is important to note that the value of W is not directly comparable across studies. In this case, the value of W=736 suggests a substantial difference in production between the seropositive and seronegative animal groups. These results may have important implications for understanding the factors affecting production in the studied context. The results of the statistical analysis showed that the 'gestation period' variables were related to seropositivity and seronegativity (P<0.05). Seropositive animals had a gestation period of 269 days compared to seronegative animals, who had 278 days. The value of W for this variable was 718, indicating a difference between the two evaluated samples.The results showed that 'abortion' variable did not have a statistically significant association with the studied seroprevalence (P-value=0.09); OR=3.77; CI:95%). This means that the null hypothesis of 'no significant difference in abortion prevalence between the studied groups' cannot be rejected. However, it is important to note that the OR value of 3.77

suggests a potential association between the presence of the studied condition and an increased risk of abortions. The average gestational age at which abortion occurred was 7.6 months, with the minimum age being 6.5 months and the maximum age being 8.5 months.

Conclusions

This study investigated the seroprevalence of *N. caninum* in a dairy cattle farm in mainland Portugal using the indirect ELISA method. The overall seroprevalence of this parasite in the farm was 28%. A significant relationship was found between *N. caninum*infection and productive and reproductive parameters, specifically in 'production' and 'gestation time'. Therefore, the implementation of prevention and control measures in farms is crucial for seroprevalence reduction.

1805 - Comparative transcriptome analysis of Babesia bigemina attenuated vaccine and virulent strains of Mexican origin

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Objectives

Bovine babesiosis, caused by the protozoan *Babesia bigemina*, is one of the most important hemoparasitic diseases of cattle in Mexico and the world. An attenuated *B. bigemina* strain maintained under in vitro culture conditions has been used as a live attenuated vaccine, however, the biological mechanisms involved in attenuation are unknown. The objective of this study was to identify through a comparative transcriptomics approach, the components of the *B. bigemina* virulent parasites that are differentially expressed in vivo, as opposed to those expressed *by B. bigemina* attenuated vaccine parasites when inoculated into naïve cattle.

Material and methods

The biological material under study was obtained by inoculating spleen-intact cattle with infected erythrocytes containing either the attenuated strain (3 steers) or a virulent field strain (3 steers). After RNA extraction, cDNA library construction and RNA-seq Next Generation Sequencing, transcriptomic analysis was performed, followed by bioinformatic Differential Expression (DE) analysis and Gene Ontology (GO) term enrichment. Filtering of the quality reads was carried out using the FastQC bioinformatics software version 0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The high-quality reads were mapped to the reference *B. bigemina* genome (GCF_000981445.1) downloaded from the National Center for Biological Information (NCBI). BWA program (v0.7.17-r1188) was used and subsequent filters were applied to separate the sequence reads that were mapped, ordered and marked as optical duplicates. A count and normalization of the mapped sequence reads were carried out using the Express program (v1.5.1). The differential expression analysis was performed using the three biological replicates for each of the *B. bigemina* strains compared in this study, using the website "IDEAMEX Integrated Differential Expression Analysis MultiEXperiment", (IDEAmex) (http://www.uusmb.unam. mx/ideamex/) applying three distinct differential expression packages: edgeR, DESeq2 and NOISeq . To report differentially expressed genes, a cut-off line of <0.01 "False Discovery Rate" (FDR) and a >2 logarithm of the fold change (logFC) was set up [1].

Results

A total of 519 differentially expressed genes were identified in the strains analyzed. In addition, GO analysis showed similarities and differences in cellular components, biological processes, and molecular functions. The attenuated strain of *B. bigemina* derived from in vitro culture presents global transcriptomic changes when compared to the virulent strain.

Conclusions

The data obtained provide information on the possible molecular mechanisms involved in the attenuation or pathogenicity for each of the strains analyzed, providing molecular markers that might be associated with virulence or potential vaccine candidates.

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PHARMACOLOGY AND TOXICOLOGY

1096 - Pharmacokinetic and Clinical Evaluation of Enrofloxacin HCl-2H2O (enro-C) for Bovine Leptospirosis

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Objectives

Bovine leptospirosis is mainly caused by the *Leptospira interrogans* serovars Hardjo Hardjoprajitno and Borgpetersenii Hardjobovis. It is a worldwide distribution and causes significant production losses, along with the constant risk of transmission to humans. Antibiotic treatments for reducing clinical signs and eliminating carrier status have demonstrated moderate efficacy. In this regard, based on the pharmacokinetic and pharmacodynamic properties of enrofloxacin hydrochloride-dihydrate (enro-C) and previous studies in animal models, its evaluation against bovine leptospirosis was considered, even though fluoroquinolones are not indicated for the treatment of the disease.

Material and methods

Thirty-six Holstein Friesian cows in six groups were used for the pharmacokinetic study. Three groups received doses of 10, 15, and 20 mg/kg of enro-C, and three other groups received a similar treatment with reference to enrofloxacin (enro-R). Enrofloxacin concentrations in serum samples collected at 1, 2, 3, 4, 6, 12, and 24 hours were determined using HPLC. Key pharmacokinetic and pharmacodynamic variables, C_{MAX}/MIC, and $AUC_{0\text{-}24}$/MIC, for the six treatment groups were incorporated into Monte Carlo simulations based on target attainment ratios C_{MAX}/MIC = 10 and $AUC_{0\text{-}24}$/MIC = 125, as functions of optimal bactericidal serum concentration.

The clinical efficacy study included 105 Holstein-Friesian cows in the geographic area A (HL), located in the Mexican highlands, and 38 F1 crossbreed Cebu x Holstein-Friesian cows in the geographic area B (TL), located in the tropics of southeastern Mexico. Patient inclusion in the study was based on a scoring system to assess the risk of leptospirosis, considering clinical signs and microscopic agglutination testing. Infection confirmation was done through PCR of urine samples. Fifty-six patients in HL and 21 in TL were treated with 15 mg/kg of enro-C (enro-C_A and enro-C_B) for 5 days, while 49 cows in HL (STR/PEN_A) and 17 in TL (STR/PEN_B) received 25 mg/kg of streptomycin plus 15,000 IU of procaine penicillin G for 5 days.

The pharmacokinetic parameters obtained from the six tested groups were analyzed using one-way ANOVA and Bonferroni tests. The difference in antibody titers before and after treatment with enro-C and streptomycin/penicillin G procaine in both geographic areas was assessed using a paired t-test. The average antibody levels were independently analyzed between treatments in each scenario through analysis of variance. A significance level of $P < 0.05$ was established.

Results

Dose increments of 10, 15, and 20 mg/kg resulted in nearly linear increases in the key pharmacokinetic parameters assessed in the enro-C and enro-R groups. A statistically significant increase in pharmacokinetic values was observed when comparing the enro-C and, enro-R groups at the same dose in all cases ($P < 0.01$).

The target attainment probabilities established for Monte Carlo simulations indicate that for *Leptospira* spp. sensitivity equal to or less than 0.5, only the doses of 15 and 20 mg of enro-C can meet the set goals.

The clinical study demonstrated a significant reduction in antibody titers with both treatments in both geographical areas ($P < 0.05$). In the enro-C_A group, 87.5% of urine samples from cows tested negative 5 days after treatment, and 92% did so 28 days after, while in the STR/PEN_A group, the values were 67.34% and 71.40%, respectively. In the enro-C_B group, 80.95% and 90.47% tested negative on days 5 and 28 after treatment, whereas in the STR/PEN_B group, 76.47% tested negative at both sampling times. The total number of PCR-negative urine samples in the enro-C_A group was statistically higher than the corresponding values in the STR/PEN_A group, both on days 5 and 28 ($P < 0.05$). However, no significant differences were observed in the enro-C_B and STR/PEN_B groups. Follow-up for 90 days after treatment indicated pregnancy rates of 86.53% and 79.06% for cows in the highland area treated with enro-C_A and STR/PEN_A, respectively, while in the tropical area, they were 88.88% and 87.5% for enro-C_B and STR/PEN_B, respectively.

Conclusions

The pharmacokinetic behavior of enro-C and its clinical efficacy indicate that a daily injection of 15 mg/kg for 5 days is highly effective in the treatment of bovine leptospirosis. These findings generate the first report of a fluoroquinolone with high efficacy in the treatment of leptospirosis in cattle and provide an option to streptomycin-penicillin G treatment.

1142 - Intake of electrolyte-enriched milk is as effective as administration of oral electrolyte solution in correcting imbalances in diarrheal calves

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Objectives

Oral rehydration is the most used method to correct dehydration and electrolyte and acid-base imbalances in calves with diarrhea. The traditional method is based on diluting the electrolyte concentrate (EC) in water, creating the oral electrolyte solution (OES). A practical alternative method, whose effectiveness needs to be proven, is the dilution of EC in milk or milk replacer offered as a meal. The objective of this study was to compare the efficacy of oral administration of a commercial EC (Glutellac; Elanco) diluted in water or milk for the reversal of water, electrolyte and acid-base imbalances in neonatal calves with induced osmotic diarrhea and dehydration.

Material and methods

24 healthy Jersey calves, 10 to 15 days old, with 28.4±3.1 kg BW were used in this randomized controlled clinical trial. The calves were subjected to induction of osmotic diarrhea and dehydration using a proven protocol: whole milk (16.5 mL/kg), sucrose (4 g/kg, diluted in a 20% solution), spironolactone (2 mg/kg) and hydrochlorothiazide (2 mg/kg) administered orally every 8 h for 48 h, and water restriction at night for 12 h. After the induction period, they were randomly distributed into two treatment groups (n=12): GM with EC diluted in milk during meals, and GW with the EC diluted in water (volume corresponding to 5% BW; 4 and 12 h). All were fed with milk (volume corresponding to 4% BW; 0, 8, and 16 h) and had free access to water. Throughout the experimental period, calves were submitted to clinical examination every 8 h. Venous blood samples were collected at the following time points: -48, -24, 0, 8, 16, 24, and 48 h. The daily volume of water spontaneously ingested was measured at -24, 0, 24, and 48 h. The following variables were measured or calculated: packed cell volume (PCV), total plasma protein (TP), pH, partial pressure of carbon dioxide (pCO_2), bicarbonate ion (HCO_3^-), base excess (BE), Na^+, K^+, Cl^-, L-lactate, creatinine, strong ion difference (SID_3), anion gap (AG), total concentration of non-volatile weak acids (A_{tot}), and percentage change in plasma volume (%PV). Data were analyzed by two-way repeated measurements ANOVA.

Results

The calves presented moderate dehydration (GM: 8.9±0.8%; GW: 9.1±0.7%), hyponatremia (GM: 133.0±6.1 mmol/L; GW: 133.6±4.3 mmol/L), relative hyperchloremia (GM: SID_3 = 36.2±2.8 mmol/L; GW: SID_3 = 36.6±3.2 mmol/L) and mild strong ion metabolic acidosis (GM: BE = -4.8±3.6 mmol/L: GW: BE = -4.8±3.7 mmol/L). Plasma volume expansion was faster in GW (P=0.014), and voluntary water intake was greater in GM (P=0.044). Both hydration methods were effective in correcting imbalances without distinction between them (P>0.05).

Conclusions: Based on these results, we can conclude that the administration of EC diluted in milk is as effective as the administration of SEO for correcting moderate dehydration in calves with induced osmotic diarrhea that have free access to water. Both treatments were able to correct mild metabolic acidosis with the same effectiveness.

1226 - Colostrum quantity and quality assessment in Italian dairy farms

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Objectives

Colostrum high quality and quantity are known to be key for a successful passive transfer of immunity and, more broadly, for calves' health during the neonatal phase. Several parameters can be measured during the colostrum production process such as the amount of colostrum produced, immunoglobulin content, bacterial load and colostrum fed. The aim of the current study was to describe colostrum production, storage and administration process in a sample of Italian dairy farms.

Material and methods

Nine Holstein herds milking twice a day and located in the North of Italy were voluntary enrolled into the study. A total of 112 colostrum samples equally distributed among farms were collected at the time of the administration to calves. Information regarding cow identification, calving date, parity, time from calving to first milking, amount of colostrum produced, type of colostrum fed (fresh, refrigerated, frozen) was collected. Just before the administration to calves, a 30ml sample was taken and analyzed with an optical Brix refractometer, as a proxy of the immunoglobulin content. The sample was then frozen and sent to the University of Perugia laboratory for microbiological analysis to determine total bacteria count (TBC) and total coliform count (TCC). Bacteria count results were log-transformed for the analysis and categorized in dichotomous variables (high/low) using the following thresholds: 100.000 colony forming units (cfu) per ml for TBC and 10.000 cfu/ml for TCC (Šlosárková et al., 2020). Finally, colostrum was administered to 82 female calves and 30 males. Calf birthweight was measured with a weight tape [Holstein dairy calf weigh tape, Nasco®] and colostrum quantity provided to each calf during the first meal was registered. Differences among parity groups, farms and storage methods were assessed using ANOVA models or contingency analysis. Simple linear regression was used to address the correlation between continuous variables. The statistical analysis was performed with JMP 16® (SAS), significant differences were declared at $P < 0.05$ and tendencies at $P < 0.10$.

Results

Dams were 33% lactation (LACT)=1 (n=37), 26% LACT=2(n=29) and 41% LACT=3+(n=46). All multiparous cows were dried-off using internal teat sealant and 72% of them received also an intramammary antibiotic treatment. Colostrum was obtained from a complete first milking occurring 337±244 minutes after calving (mean and standard deviation) and the amount produced was 6.06±2.39 liters, with 13.4% of cows giving less than 4 liters. There were no significant differences among parity groups. Average Brix values were 25,5±3.1 with 14.3% of samples below 22, a commonly used threshold for good quality colostrum. Significant differences were found among farms, with farm mean Brix values ranging from 22.00 to 27.33. Sixty-seven percent of colostra were frozen (n=64), 31% was used fresh (n=35) and 12% (n=13) was refrigerated before administration. Log-TBC was 11.37±1.48, with 42% of high samples. Log-TCC was 8.70±2.18 with 40% of samples having high counts. The proportion of high samples for TBC tended to be greatest for frozen samples (48.4%) followed by fresh colostrum (40.0%) and refrigerated (16.7%) (p=0.09). TCC high counts differed depending on the

storage method, with fresh colostrum having the greatest proportion of high counts (60.0%), while refrigerated had 38.5% and frozen 29.7% (p=0.01). The correlation between log-TBC and log-TCC was significant (p<0,0001), but weak, with an r^2 of 0.16. Overall calf birthweight was 41.14±3.81 and colostrum administered was 3.56±0.57 Kg. In 78.2% of the calves the amount of colostrum fed did not reach 10% of bodyweight.

Conclusions

This descriptive study demonstrated that, within the current farm sample, colostrum produced is not always adequate and colostrum fed is often insufficient. Moreover, quality in terms of Brix and microbial contamination represent a weakness of the process requiring a more intense screening program, together with continuous education on dry cow management and good hygiene practices for colostrum handling directed to farmers and their employees.

References

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1235 - EFFECT OF VACCINATION WITH A MULTIVALENT MARKER VIRAL VACCINE AGAINST RESPIRATORY DISEASE IN A COMMERCIAL FEEDLOT.

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Objectives

BRSV is a major cause of respiratory disease in cattle beside of being one of the primary viruses involved in the Bovine respiratory disease (BRD) complex, the most prevalent cause of morbidity among feedlot cattlee. One of the best strategies to achieve prevention is through vaccination. A multivalent marker vaccine (DIVENCE PENTA, HIPRA) against BRSV, BoHV-1, PI-3 and BVDV-1 and BVDV-2 has been recently developed. The objective of this randomized, blinded and negative-controlled field trial was to evaluate the clinical efficacy of vaccination with DIVENCE PENTA when administered to calves under field conditions in a commercial feedlot.

Material and methods

One batch of 109 Friesian male calves entered onto a commercial feedlot farm with standard management and husbandry conditions. Animals were randomly distributed in the two study groups when the average age of the batch was 10-week-old (ranging from 61-day to 16-week-old) (study day D0). Animals from the DIVENCE PENTA group (n= 54) were vaccinated with the DIVENCE PENTA vaccine and animals from the Control group (n= 55) received a placebo (Phosphate Buffered Saline, PBS). Both groups were administered two intramuscular doses (2ml/dose) of the corresponding product 21 days apart. Incidence of cases of respiratory disease, severity of clinical signs, number of antimicrobial treatments due to respiratory disease and mortality were blindly recorded from vaccination (study day D0) until the end of the fattening period (overall follow-up). A score was given to all respiratory disease cases using a 4-point scale to evaluate the severity of clinical signs (0: clinically normal; 1: mild signs of depression and mild respiratory signs present; 2: moderate signs of depression and moderate respiratory distress; 3: severe signs of depression and sever respiratory distress; 4: moribund). An outbreak was considered when more than 10% of animals showed signs of respiratory disease for 2-3 consecutive days (MAPA, 2023). A five-day monitoring was then conducted (outbreak follow-up). Blood and nasal exudate samples from the reported cases were also collected. The statistical analysis was done using the R software v3.1. A *p-value* < 0.05 was chosen as the limit for statistical significance. Logistic regression analysis was performed for groups comparison for binary response variables while Wilcoxon test for quantitative variables.

Results

A BRSV outbreak was reported 23 days after the start of the vaccination program. Laboratory results and clinical signs observed confirmed BRSV as the only etiological pathogen responsible for the outbreak. A significant reduction of 62.1% in the vaccinated group was observed on the incidence of respiratory disease cases during the outbreak follow-up ($p < 0.001$). Vaccinated group significantly reduced ($p < 0.001$) incidence from 53.7% to 20.4%, odds ratio: 0.22 (0.09-0.52). Severity of respiratory clinical signs was also significantly lower in the vaccinated group compared to the

control group (*p*= 0.02). The mortality rate was numerically reduced by 67%, being lower in the vaccinated group (1.85%) in comparison to the control group (5.56%).

A 10-month follow-up period from first vaccination to slaughter was carried out. On average, animals were 303 days on feed from the 1st vaccination dose to slaughter. Results on the incidence of respiratory disease (at least one case of respiratory disease) and total number of respiratory cases across the fattening period were 57.41% and 1.24 in the control group and 33.33% and 0.48 in the vaccinated group. A significant reduction of 41.9% on the incidence of respiratory disease in the vaccinated group was observed. Specifically, vaccinated group significantly reduced ($p < 0.01$) infection from 57.4% to 33.3%, odds ratio: 0.37 (0.17-0.81). As for antibiotic treatments during the overall fattening period, the total number of calves that required one or more than one antibiotic treatment for respiratory disease was significantly lower in the vaccinated group compared to the control group. A reduction of 41.9% and 56.3% in the usage of antibiotic treatments was observed in the vaccinated group for at least one treatment or more than one, respectively. No statistically significant differences between groups were observed on the mortality rate, although mortality was numerically reduced by 75% in vaccinees across the whole fattening period (7.41% control vs 1.85% vaccinee).

Conclusions

Vaccination with DIVENCE PENTA significantly reduced the morbidity and severity of clinical signs associated to respiratory disease, during a respiratory outbreak and during the entire fattening period. The usage of antimicrobial treatments was also reduced. In addition, vaccination also reduced numerically the mortality across the study.

1256 - Pelvic limb paralysis outbreak in bovine cattle due to consumption of Melochia pyramidata in Jalisco, Mexico

Author: Jorge Luis García Valle, Johnatan Ruíz-Ramírez, Rafael Macedo-Barragán, Daniel Armando Topete Bustamante, Tiburcio Lizama-Munguía, Rafael Ramírez-Romero, Luis Jorge García-Márquez

Objectives

The objective of the present study was to describe pelvic limb paralysis in bovine cattle due to consumption of *Melochia pyramidata* in Jalisco, Mexico.

Material and methods

Eight cases of pelvic limb paralysis were registered from August 23 to September 28, 2023 in a livestock production unit located in Pihuamo, Jalisco, Mexico (18°57'30" - 19º 23' 30" N and 103°10'00" - 103º32'05" W) which were initially diagnosed as bovine paralytic rabies. Seventy crossbreed Holstein cattle grazed on African Star-grass (*Cynodon nlemfuensis*) where in addition, evidence of consumption of “Purple Huinar” (*Melochia pyramidata*), a plant characterized by its content of Melochinin (pyridinic alkaloid) was found. Clinical signs presented progressively and over a month of evolution included difficulty of raising the pelvic limbs during walking, incoordination (ataxia), paralysis of the hind limbs (drooping), prostration and death. From a sick animal, 5 ml of blood was collected in a vacutainer tube without anticoagulant and a blood smear was performed. In addition, a necropsy of a male bovine was performed in the Laboratory of Pathology of the University of Colima. The organs were fixed in neutralized formalin at pH 7.0 and 10%, the tissues were processed with the histological technique and were cut at 5 µm and stained with Hematoxylin-Eosin (HE) and Luxol Fast Blue (LFB) and a half of the cerebral hemisphere was refrigerated for immunofluorescence

Results

Biochemical study revealed a marked increase in CK and AST due to muscular effort, panhypoproteinemia with hypocalcemia related to lipoalbuminemia due to loss and apparent liver injury, hyponatremia and hypochloremia due to hypotonic dehydration. Negative results for *Babesia* sp., *Anaplasma* sp. and rabies. The necropsy findings were edema in the subcutaneous tissue (anasarca) and presence of white-translucent fluid in the thoracic and abdominal cavities (hydrothorax and ascites) and in the pericardial sac (hydropericardium). Pericardial and perirenal adipose tissue with serous atrophy of fat, dilation of the right ventricle, pale liver with rounded edges, sciatic nerves with edema and hemorrhages. Histological lesions were present in the neuropile of the central and peripheral nervous system, where clear vacuoles corresponding to demyelination (spongiosis) and tortuous and swollen axons (axonal degeneration) were observed. In the ventral portion of the spinal cord, the white matter showed areas similar to those described in the cerebellum and medulla oblongata with tortuous, swollen peripheral nerve axons (axonal degeneration) and clear vacuoles (demyelination). Demyelination was evident with LFB, in myocardium it was observed with loss of its striations and vacuolization of the sarcoplasm (necrosis), presence of *Sarcocystis* sp., in cardiac and skeletal muscle, interstitial neutrophilic inflammation in skeletal muscle and moderate multifocal hepatic coagulative necrosis.

Conclusions

Consumption of *M. pyramidata* by cattle is common in the tropical areas of Mexico, causing nervous signs, confusing it with bovine paralytic rabies. The description of this case can guide the veterinary clinician in the identification of the toxic flora, signs, lesions and consider it as a differential diagnosis of diseases that affect the nervous system of bovines.

1304 - Assessing gastrointestinal egg excretion and milk parameters in dairy sheep in Austria

Author: Floriana Sajovitz , Johanna Schachermair, Isabella Adduci, Shi Yan, Sandra Wiedermann, Alexander Tichy, Anja Joachim, Thomas Wittek, Katharina Lichtmannsperger

Objectives
Gastrointestinal nematode (GIN) infections can have a major impact on sheep performance and may lead to substantial economic losses in farming. Various methods are used to target sheep being affected by a GIN infection, such as the FAMACHA© score, body condition score or faecal egg count. In Austria, the national dairy sheep milk recording association provides performance data for each individual sheep through the whole lactation. Sheep farmers can subsequently monitor milk yield and milk constituents (fat, protein, urea) over the course of lactation. Studies have shown that there is a correlation between the excretion of gastrointestinal nematode eggs and milk yield and milk constituents in sheep. This could be a further step towards the early detection and monitoring of sheep infected with gastrointestinal nematodes.
Material and methods
In order to investigate whether there is a correlation between faecal egg excretion and milk yield and milk constituents parameters in Austria, 708 dairy ewes from 10 sheep farms were included in the study. Individual egg excretion was analysed using the Mini-FLOTAC method. Milk yield, milk protein and milk fat concentrations were used from five consecutive measurements, with the sheep being under 100 DIM at the first measurement. The Spearman rank correlation coefficient was used for the statistical calculation of the correlation between the milk performance data including milk yield (kg), milk fat (%), milk protein (%).
Results
The results showed a correlation coefficient of r= 0.036 (p=0.043) for milk yield, r= -0.199 (p= 0.000) for milk fat content (%) and r= -0.035 (p= 0.047) for milk protein content. The quotient of milk fat and milk protein showed a negative correlation with the EpG (r= -0.251; p= 0.000).
Conclusions
The results of the study show only a weak correlation between egg excretion and milk performance parameters. The strongest relationship was between the EpG and the milk fat and milk protein quotient. However, other non-parasitic influences must be taken into account (breed, age, feeding, pathogens). It would be beneficial to repeat the study at a different time point (during or after the main grazing season) or to include more faecal sampling points.

1306 - Pharmacokinetic profile evaluation of isometamidium (Trypamizol®) in cattle in Brazil

Author: Daniel Rodriques, Tom Strydom , D.M.B Zapa, L M Heller, Lidia Mendes de Aquino, WDZ Lopes, Luis Vettorato, Francisco Barufi, Heitor Amaral

Objectives
To evaluate the pharmacokinetic profile of isometamidium (Trypamizol® (MSD Saúde Animal) following its intramuscular administration at 1 mg/kg body weight in cattle.
Material and methods
Six isometamidium-naïve calves (three males and three females) were selected for the study. Animals were acclimatized for 4 days (Day -4 to Day -1) before treatment administration. On Day -4, the animals were identified, weighed, and submitted to clinical examinations. The animals were kept under the same environmental conditions throughout the experimental period, with natural light and temperature and not coming into physical contact with other non-study animals. Trypamizol® was administered to the calves once on Day 0, by deep intramuscular injection, at a dose rate of 1 mL per 40 kg body weight. Plasma samples from each calve was collected before treatment on Day 0 and at the following timepoints post-treatment: 1, 3, 6, 9 and 12 hours and 1, 2, 7, 14, 21, 28, 42, 56 and 68 days post-treatment. The samples were analyzed using the mass spectrometry technique coupled to an ultra-performance liquid chromatograph (LC-MS/MS) for the quantification of isometamidium chloride in plasma followed by calculation of pharmacokinetic parameters.
Results
After intramuscular administration of Trypamizol® to calves, a Cmax of 488.57 µg/L isometamidium chloride was detected at a mean Tmax of 1 hour after administration. A plasma half-life of 10.46 hours and an area under the plasma concentration curve [AUC 0-t (µg/L*h)] of 2889.12 µg/L*h was detected.
Conclusions
The Cmax of 488.57 µg/L Trypamizol® detected at an average Tmax of 1 hour after administration allows for 100% efficacy on trypomastigotes present in the bloodstream.

1318 - Development of a novel combined biosecurity risk assessment scoring tool and cattle movement audit

Author: Siobhan O'Donovan, John Mee F, Luke O'Grady, Conor McAloon, Tim Geraghty

Objectives

Current biosecurity tools lack inclusion of cattle movement audits and instead rely on farmer or veterinarian (from memory) responses to questionnaires. However, cattle movement is the most important route of transmission of infectious pathogens. Hence, the objective of this research was to develop a new biosecurity risk assessment tool that incorporated a cattle movement audit as phase one of a larger study investigating biosecurity risks and practices on Irish dairy farms.

Material and methods

A biosecurity questionnaire with 75 questions was drafted based on the peer-reviewed literature. The response options to questions were weighted by expert opinion using the best-worst scaling (BWS) system in the software Conjointly. The BWS allocates scores to each question to produce an overall score. Experts in biosecurity carried out the scoring process in three online sessions. The five expert groups included veterinary practitioners, ECBHM (European College of Bovine Health Management) members, The Department of Agriculture Food and Marine (DAFM) specialists in biosecurity, Animal Health Ireland's (AHI) Technical Working Group for biosecurity and the project team. Each group was given a tutorial on how the software assigned scores. The experts assigned "best" and "worst" to multiple biosecurity practices. For example*, "Clinical disease outbreaks are always investigated"* was considered "best" practice, while "*Clinical disease outbreaks are never investigated*" was ranked the "worst" management practice. In addition, cattle introduction indicators were developed; in degree, inward strength and secondary inward degree. In degree was defined as the number of cattle moved onto the farm in the previous 3 years, inward strength was defined as the number of herds from which cattle were introduced onto the farm in the previous 3 years, and secondary inward to degree extended this to the herds from which source herds introduced cattle from.

Results

Following completion of the three online sessions with 37 experts the software assigned best and worst scores to 53 biosecurity practices and an overall farm score. These scores highlight areas of highest risk - moderate risk – lowest risk. A traffic light system of red, amber and green is used to illustrate such risk factors for farmers. Similarly, the cattle introduction audit is used to assign a risk category for each herd based on its cattle movement patterns. In phase two of this study this new biosecurity toll will now be tested in a large-scale field study of biosecurity risks and practices on Irish dairy farms.

Conclusions

This novel approach to assigning biosecurity scores harnesses the merits of both expert opinion and conjoint analysis. The inclusion of a cattle introduction audit completes the risk assessment of the biosecurity status of a dairy farm

1343 - BLOOD TRANSFUSION IN HEIFERS WITH SUSPECTED OUTBREAK OF CLOSTRIDIOSIS: CASE REPORT

Author: João Silva, Paulo Capêlo, André Martins, Paula A. Oliveira, Ana Claúdia Coelho

Objectives

This study aims to report two clinical cases where blood transfusion was used in Holstein-Friesian heifers with clinical signs compatible with anaerobic infection (identical to clostridiosis). Blood transfusion is recommended in ruminants in situations of hemorrhage or erythrolysis associated with traumatic bleeding, parasitism, or immune-mediated anemia (Balcomb & Foster, 2014).

Material and methods

Veterinarians from the region of Barcelos, Portugal, were called to analyze the death of a heifer from a herd of 10 heifers aged between 7 and 8 months. On arrival at the farm, it was confirmed that one animal had died, with two others having difficulty standing and moving. The remaining animals showed no clinical signs of illness.

A complete physical examination was carried out on the two weakest animals and identified tachypnea, rectal temperature below 38.0ºC, difficulty maintaining sternal decubitus and standing, tympanism, abdominal pain, and blindness.

The dead animal had red-colored fluids coming out of the orifices (nostrils, mouth, eyes, and anus) and was abdominally distended.

Taking into account the examination of the dead animal and the clinical signs shown by the other animals, the presumptive diagnosis was anaerobic infection, probably clostridiosis, with differential diagnoses of bacterial meningitis or food poisoning.

Treatment of the two weak calves consisted of intravenous (IV) fluid therapy with ringer's lactate (2L), hypertonic saline (0.5L), butylescopolamine bromide (0.4 mg/kg, IV), metamizole sodium monohydrate (40 mg/kg, IV). A combination of benzylpenicillin procaine and dihydrostreptomycin (1.5 mL per 25 kg p.v.) was also administered intramuscularly. Five hours after the initial treatment, the animals were observed and showed signs of neurological alterations.

Given the unfavorable evolution, it was decided to transfuse whole blood. The blood donors were two cows from a different farm, vaccinated 22 days previously with *Clostridium perfringens* toxoid type A (α), *Clostridium perfringens* toxoid types B and C (β), *Clostridium perfringens* toxoid type D (ε), complete inactivated culture of *Clostridium chauvoei, Clostridium novyi* toxoid, *Clostridium septicum* toxoid, *Clostridium tetani* toxoid, *Clostridium sordellii* toxoid, and *Clostridium. haemolyticum* toxoid.

Blood transfusion bags containing citric acid, sodium citrate, sodium phosphate monohydrate, dextrose monohydrate, and adenine (KRUUSE® CPDA-1) collected the blood. Two 500 mL bags were collected, and 500 mL was administered to each sick calf. 12h after the transfusion, the animals were stable, ruminating, with a rectal temperature of 38.5ºC and 38.7ºC. Treatment with benzylpenicillin procaine and dihydrostreptomycin was continued for five days. The remaining seven heifers with less severe clinical signs had abdominal distension and few rumen movements, so intramuscular administration of the same combination of antibiotics was recommended for five days.

In addition to the veterinary care provided, it was recommended that the diet be changed from corn silage and feed to straw and wheat bran during antibiotic therapy.

Once this was complete, it was suggested that the diet be gradually adjusted to the quantities and raw materials used before the outbreak.

Results

Despite the presumptive diagnosis and the very delicate prognosis in these situations, none of the seven calves treated died. The two calves that were in the worst condition, 12 hours after the blood transfusion, still had some neurological signs, such as blindness. Still, they gradually disappeared over the days and recovered their vital functions seven days later.

Conclusions

Restraining the donor animals for blood collection did not affect their well-being or put their health at risk because the method is minimally invasive, involves placing a catheter in the jugular vein, and the volume of blood collected is within the range of what is considered normal (Balcomb & Foster, 2014).

For the outcome of these clinical cases, blood transfusions were fundamental to the success of the treatment and consequently reversing the clinical signs compatible with clostridiosis, allowing the animals to survive.

Balcomb, C., & Foster, D. (2014). Update on the Use of Blood and Blood Products in Ruminants. Veterinary Clinics: Food Animal Practice, 30(2), 455–474. https://doi.org/10.1016/j.cvfa.2014.04.001

1351 - Pharmacokinetics of transdermal flunixin meglumine in hair and wool sheep

Author: Danielle Mzyk, Ronald Baynes, Jennifer Halleran

Objectives

Although there is evidence to suggest that some available drugs relieve pain in livestock species, the lack of regulatory approval by the U.S. Food and Drug Administration may impede practical on-farm application for sheep producers and veterinarians. Transdermal flunixin is the only drug approved by the United States Food and Drug Administration to control pain in a ruminant species. Specifically, transdermal flunixin is approved for the control of pain associated with footrot in cattle. To date, there are no pharmaceutical pain relief options approved by the US FDA specifically for use in sheep. Furthermore, obtaining such approval remains challenging as drug efficacy must be proven. Pharmacokinetic studies of transdermal flunixin have been reported for dairy cows (Gorden et al., 2019; Kleinhenz et al., 2016), lactating dairy goats (Meria et al. 2022), and meat goats (Reppert et al., 2019), but no studies have been completed in sheep. The objective of this pilot study is to determine plasma pharmacokinetic parameters of transdermal flunixin meglumine in wool vs hair sheep.

Material and methods

4 ewes (dorsets [n=2]; Katahdin [n=2]) were administered 3.3 mg/kg of transdermal flunixin meglumine on dorsal topline once. Plasma samples were collected over 96 hours and analyzed by tandem mass spectrometry (UPLC-MS-MS).

Results

Following transdermal administration, flunixin was well absorbed across both breeds. Using non-compartmental pharmacokinetic analysis, the mean area under the curve extrapolated to infinity was 15.7 ± 1.3 µg x h/mL. Flunixin reached peak concentrations (Maximum average concentration was 1.1 µg/mL) in plasma between 4 and 6 hours after application. Plasma terminal half-life was 16.7 ± 2.2 hr. Mean residence time was 14.3 h after topical administration.

Conclusions

Results from this study demonstrated that flunixin administered to hair or wooled sheep as a topical preparation reached plasma concentrations similar to those reported in cattle, and almost 10x higher than reported in Boer goats. The data produced from this study on flunixin in wool and haired sheep concludes that flunixin is rapidly absorbed and has a shorter half-life compared to previous reports in goats. The overall outcome of this study will provide a starting point to establish a safe and effective therapeutic dosing regimen for the treatment of pain and inflammation in sheep.

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4. Reppert EJ, Kleinhenz MD, Montgomery SR, Bornheim HN, Magnin G, Sidhu PK, Zhang Y, Joo H, Coetzee JF. (2019) Pharmacokinetics and pharmacodynamics of intravenous and transdermal flunixin meglumine in meat goats. Journal of Veterinary Pharmacology and Therapeutics. 42(3): 309.

1413 - EPIDEMIOLOGICAL CHARACTERISTICS OF RHIPICEPHALUS MICROPLUS IN URUGUAY

Author: Ulises Cuore Passeggi, Leandro Tejera

Objectives

The goal of this study is to collect information on R. microplus generated in different ecosystems of Uruguay.

Material and methods

The methodology used for ecological studies was based with the same method of Harley, 1966.

Tick population: The population of R. microplus used in all exhibitions was the so-called "Mozo" strain that has been maintained in the DILAVE “Miguel C. Rubino” since 1973 as a reference tick population susceptible to all acaricides.

Geographic location of exhibitions: Depending of the trial, teleogyne exposures will take place every 15 or 30 days. 3 to 5 fully engorged teleogines were placed in bronze mesh tubes with 80 threads per inch (Photo 1). 3 to 5 replicates were carried out at a time, the tubes were placed on the soil surface covered by a thin layer of grass. Observations were made every 15 or 30 days.

In the Departments of Canelones, Artigas, Tacuarembó and Cerro Largo, the exhibitions were held under the open sky. While in Paysandú, Salto, Lavalleja and Rivera, a comparative study was carried out between open-air exposures and under protection of natural or implanted forest.

Experimental design: Depending of the trial, teleogyne exposures will take place every 15 or 30 days. 3 to 5 fully engorged teleogines were placed in bronze mesh tubes with 80 threads per inch (Photo 1). 3 to 5 replicates were carried out at a time, the tubes were placed on the soil surface covered by a thin layer of grass. Observations were made every 15 or 30 days.

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Data logging: The following data were recorded regarding the behavior of the Non-Parasitic Cycle:

Protochy: Period between the fall of the female and the beginning of oviposition.

Incubation period: Beginning of lay until beginning of erosion.

Larval longevity: From the beginning of hatching until the death of 100% of the larvae.

Criterion to estimate a tick generation: It was based on the development, permanence, and number of available larvae, which will be responsible for forming the next generation.

Weather records: The data were obtained from the meteorological stations of the Meteorology National Director, in the same place where the presentations were made.

Results

With the information generated, the duration of the Non-Parasitic Cycle (NPC) and the number of generations of ticks per year were estimated.

- The minimum protocol period was 2 days and a maximum of 90 days.
- The minimum incubation period was 30 days and maximum 100 days.
- The minimum larval longevity was 15 days, and the maximum was 6 to 10 months.
- The Non-Parasitic Cycle can last between 8 and 13 months at most.
- The average number of generations developed per year is 3, obtaining atypical results with 1.5 generations in 2007 in the Department of Lavalleja and a maximum of 4 in 2003 under natural forest protection in Paysandú.

The ecological results are dependent on the year factor mainly related to temperature and humidity factors. In 2003 and 2005, the non-parasitic cycle was not interrupted, while in 2004, during the months of June to August, the teleogines did not prosper in their reproductive stage, completely interrupting the non-parasitic cycle.

Conclusions

Despite being in a marginal area for development, R. microplus manages to reproduce efficiently throughout the country, presenting greater affinity for certain ecological areas and with better development in the northern area above the 32nd parallel.

1502 - EVALUATION OF INHIBITION OF TEA TREE OIL, CARVACROL AND THYMOL AGAINST STRAINS ISOLATED FROM CLINICAL CASES OF METRITIS

Author: Luis Fernando Espinosa Castillo, David Quintanar Guerrero, Susana Mendoza Elvira, Héctor Jimenes Severiano, Laura Hernández Andrade

Objectives

To evaluate the inhibitory effectiveness of tea tree, carvacrol and thymol (Memar et al., 2017) as natural alternatives for the treatment of different pathogens causing uterine infections.

Material and methods

A total of 14 field strains (4 of *Staphylococcus aureus*, 4 of *Streptococuss spp*, 3 of *Truperella pyogenes*, 1 of *Klebsiella spp*, 2 of *Escherichia coli*, 1 of *Proteus mirabilis*) were used to evaluate the inhibition produced by Tea Tree, Carvacrol and Thymol oils. The strains were isolated from cases of metritis in the Tizayuca Hidalgo basin (IAȘI & Sadoveanu, 2004).

Sensidiscs were prepared with filter paper of 5mm porosity and sterilized. These were impregnated with 20µl of each oil (carvacrol at 40mg/ml, thymol at 50mg/ml, tea tree at 25mg/ml) at 80%, 75% and 50% for each oil and left to rest for 12 h in refrigeration to allow impregnation.

Susceptibility testing was performed using Müller-Hilton agar for *Staphylococcus*, *Klebsiella*, *E. coli* and *Proteus* strains for their growth and Sangre agar plates for *Streptococcus* and *Truperella* strains. These were diluted in Mcfarland's 0.5 saline solution and adjusted to 600 nanometers in the spectrophotometer for sow.

Sensidiscs impregnated with the afore mentioned oils and one of ceftiofur were placed as a control antibiotic to evaluate growth inhibition; the experiment was carried out in triplicate to make its statistical evaluation. The inhibition halos were measured, and the data were stored in Excel to be evaluated by the STATA program using an ANOVA test for group comparison.

Results

The results showed that the oils with the highest halo of inhibition compared to ceftiofur were, firstly, those generated by 80% carvacrol in Gram-positive and negative bacteria, secondly, 80% tea tree in Gram-positive and negative Bacteria and thirdly, 80% thymol in Gram-positive bacteria. The sensidiscs of the three 75% oils presented halos of equal size to the control, having more effect against Gram-positive microorganisms. Finally, tea tree and 50% carvacrol sensidiscs had halos of equal size to the control for Gram positive, and those of thymol at 50% had smaller halos compared to the control for both Gram positive and negative; All results had a value of $p \geq 0.05$.

Conclusions

The use of natural oils has been shown to have an effect against the growth of the microorganisms most frequently found in cases of metritis. This may be an option against these microorganisms and a possibility to reduce the use of antibiotics and the generation of resistance.

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1505 - PHARMACOKINETICS AND MILK PENETRATION OF DOXYCYCLINE AFTER INTRAVENOUS AND INTRAMUSCULAR ADMINISTRATION TO LACTATING GOATS

Author: José Martínez, Pedro Marín Carrillo, Elisa Escudero, María Teresa Yuste, Verónica Hernandis, Elena Badillo

Objectives
The purpose of the study was to determine the pharmacokinetics and milk penetration of doxycycline (DOX) in clinically normal lactating goats (n=6) after intravenous (dose: 5 mg/kg) and intramuscular (dose: 20 mg/kg) administration.
Material and methods
Doxycycline concentrations were determined by high performance liquid chromatography with fluorescence detection. An isocratic method was used with a flow rate of 1.0 mL/min. Doxycycline eluted at approximately 9.1 minutes. The fluorescence detection was performed at an excitation wavelength of 380 nm and an emission wavelength of 520 nm. The concentration-time data were analyzed by non-compartmental pharmacokinetic methods.
Results
After intravenous administration, plasma half-life ($t_{½lz}$) and mean residence time (MRT) of doxycycline were 2.58 and 2.72 h, respectively, while after intramuscular injection significantly longer times were obtained (43.86 and 57.49 h, respectively). Bioavailability after IM administration was 61.8 ± 30.2 %. Milk concentrations run parallel to those in plasma, being the $t_{½lz}$ and MRT after IV administration of 3.19 and 5.48 h and after IM injection of 26.86 and 40.59 h, respectively. Peak concentrations (C_{max}) of doxycycline in the milk after each treatment were reached at similar times (3 and 4.8 h, respectively) but DOX concentrations were detectable until 24 and 72 h after IV and IM administration, respectively. Ratios of area under the milk/plasma DOX concentration-time curves from 0 to infinity time ($AUC_{milk/plasma}$) after IV and IM administrations were 0.37 ± 0.13 and 0.56 ±0.30, respectively, and peak concentrations ($C_{max\ milk/plasma}$) were 0.10 ± 0.05 and 0.50 ± 0.15, indicating a greater penetration into the udder after the IM treatment, possibly due to saturation of the elimination process caused by the higher dose used.
Conclusions
The intramuscular administration at the selected dose provided adequate concentrations and enlarged doxycycline disposition both in plasma and milk of goats. The study presented here clearly demonstrate that lactating goats show a different disposition parameter from non-lactating animals (Abd El-Aty et al., 2004; Turk et al., 2020) that have to be taken into account to design specific dose-regimens. PK-PD study against udder specific pathogens remain to be determined.

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1514 - PHARMACOKINETICS OF DOXYCYCLINE AFTER INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION TO SHEEP

Author: José Martínez, Elisa Escudero Pastor, Verónica Hernandis, María Teresa Yuste, Elena Badillo, Pedro Marín

Objectives

The purpose of the study was to determine the pharmacokinetics of doxycycline in clinically normal Montesina sheep (n=6) after intravenous (dose: 5 mg/kg) and subcutaneous (dose: 20 mg/kg) administration.

Material and methods

Doxycycline (DOX) concentrations were determined by high performance liquid chromatography with fluorescence detection. An isocratic method was used with a flow rate of 1.0 mL/min. Doxycycline eluted at approximately 9.1 minutes. The fluorescence detection was performed at an excitation wavelength of 380 nm and an emission wavelength of 520 nm. The concentration-time data were analyzed by non-compartmental pharmacokinetic methods.

Results

DOX concentrations were detectable until 24 and 96 h after single IV and SC dose administration, respectively. After IV injection, apparent volume of distribution calculated by the area method (V_z) and at steady state (V_{ss}) were 1.08 ± 0.57 and 0.75 ±0.25 L/kg, respectively, indicating good penetration and distribution into the body but in a lesser extent than in previous studies (Castro et al., 2009). The compound has a high lipophilicity, and extensive tissue distribution would be expected, likely with tissue binding and/or intracellular penetration. Half-life and mean residence time (MRT) values by this route were 4,03 ± 3.14 and 3.96 ± 2.31 h, respectively. The half-life was shorter than those recorded in sheep (7.02 h; Castro et al., 2009), cows (24.8 h; Ziv and Sulman, 1974), ruminant and pre-ruminant calves (14.8 and 9.88 h; Riond et al., 1989) but similar to non-lactating goats (4.62 h; Abd El-Aty et al., 2004).

DOX pharmacokinetics after SC administration are first described here. After this route the half-life and MRT were significantly enlarged (38.85 ± 10.97 and 55.69 ± 14.48 h, respectively), but longer values have been reported by Castro et al (2012) of 77.8 ± 28.4 and 91.1 ± 40.8 h, respectively, after intramuscular administration in the same species. Peak plasma concentration and time to reach maximum plasma concentration were 1806.9 ± 422.1 µg/L and 2.8 ± 1.1 h, respectively, indicating a similar extent of absorption but a slower rate than when administered intramuscularly (2.792 ± 0.791 µg/mL, 0.86 ± 0.47 h; Castro et al., 2012).

Bioavailability after SC injection was 67 ± 25 %, higher than those reported in sheep after oral administration (35.8 %; Castro et al., 2009), no data are available for IM administration in these species.

Conclusions

Results indicated that doxycycline had prolonged absorption and elimination in sheep after SC administration. A dose of 20 mg/kg would be useful to reach effective plasma

concentrations against sensible pathogens, but PK/PD remains to be determined against specific ones and the best dosing regimen in each case.

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1592 - Preparation of a topical emulgel based on thymol and tilmicosin for the treatment of bovine mastitis.

Author: Lysett Corona-Gómez, Laura Hernández-Andrade, Israel Daniel Ricardo-Gónzalez, David Quintanar Guerrero, Susana Mendoza Elvira

Objectives

Develop and characterize an emulgel system based on tilmicosin and thymol that can greatly help treat bovine mastitis.

Material and methods

Preparation of the emulgel. The aqueous phase (poloxamer + permeation agent) was kept stirring and the oil phase (Thymol + Tilmicosin + Medium Chain Triglycerides) was added little by little until completely incorporated (both phases were at 40 °C). Stirring is continued until room temperature.

Rheological evaluation and stability of the emulgel. Color, odor, texture, and consistency were evaluated, pH, specific gravity, Z potential, particle size, viscosity, stability at room temperature and accelerated stability were measured for a period of 6 months. For all measurements, measures of central tendency and the ANOVA test were used to evaluate if there were significant statistical differences.

Results

During the observation of the emulgel, for a period of six months, there were no organoleptic changes, syneresis and phase separation. After the physical stability test, no alterations were observed in its organoleptic characteristics. With respect to pH, a slight decrease was observed from the third month, remaining stable during the following three months, with acceptable values to avoid the risk of skin irritation. The oxidation process and the formation of oxidation products in the oil phase could be the reason for the slight reduction in pH values. The result of the specific gravity can be observed a slight increase that when performing a statistical test on the data indicates that there is no significant difference $p>0.05$, during a period of 6 months at room temperature, the slight increase could be attributed to the loss of water through evaporation. The stability of the emulgel for 6 months was an average of 43.3±0.5; This is because the poloxamer acts by creating a steric effect, thereby preventing the coalescence of the particles. A particle size of 1444±128.08 nm was obtained with a coefficient of variation of 2.42%, which indicates little coalition of globules for 6 months, in addition to an average polydispersity of 0.7±0.05. Viscosity values observed from week 1 to week 12 that were maintained at room temperature (28 ° ± 2 ° C) it is observed that it tends to decrease slightly from the third month onwards, this is possible because the gel matrix cannot maintain its goo. During the study time of the stability of the semi-solid system (30 days) at room temperature, no change was observed as described in the stability table.

Conclusions

The results of the rheological and stability evaluation indicated that the emulgel is stable over time for 6 months, which indicates that it is a good alternative for controlling bovine mastitis.

1754 - In situ acaricidal effect of polar extract plant against Rhipicephalus microplus

Author: Dumar Alexander Jaramillo Hernández, Angélica E. Gonzalez Reina, Natalia Pedraza Castillo

Objectives

The aim was assessment in situ acaricidal activity of methanolic extract *Momordica charantia (Mc)*, ethanolic extract *Megaskepasma erythrochlamys (Me)* and acetone extract *Gliricidia sepium (Gs)* againts *Rhipicephalus microplus* (Rm)

Material and methods

Collection of plants and elaboration of extracts. Leaves of *Mc* L. N°COL 580477, *Me* Lindau N°COL 575462 y *Gs* (Jacq.) Kunth ex Walp N°COL576461; were collected between the months of April and July, on the farm of Universidad de los Llanos, located on rural area Barcelona, Villavicencio (Meta). 4° 04'32,91" North latitude , 73° 35'02,27 West Latitude, altitude 386 MASL, 3500 mm/year precipitation, 87% HR and average ambient temperature 27°C. The plant material was treated according to the methodology of the phytochemicals analysis proposed by Sanabria (1), thus: it was dried in a circulating air furnace at 40 °C for 72 h and subsequently ground to pulverization for the elaboration of the extracts were weighed 150 g of dry plant material ground of *Mc, Me* y *Gs*; subsequently exposed to 600 mL of Methanol 99.8%, Ethanol 99.8% y Acetone 70% (Sigma-Aldrich, St. Louis, MO), respectively; each in a glass decanting funnel, for 72 hours, carrying out the continuous percolation technique to exhaustion. The percolation liquid was filtered and concentrated at 40°C in a rotary evaporator IKA® RV10 control, at reduced pressure, eventually taking to dryness in water bath (bain-marie). The extracts were transferred to amber glass vials and stored at 4°C until the time of testing, the average yield of the extraction process was 5%, subsequently, preliminary phytochemicals analyses of the extracts were performed to determine the presence of secondary metabolites through the colorimetric and CCD technique.

in-situ testing. The Acaricide activity of Eme y EMc was evaluated in Barcelona´s dairy herds, located in Km 12 runway Puerto López in Villavicencio, Meta. The evaluation of the EGs was carried out in the Agroindustry Center of the Meta "Hachón" (SENA), located in Km 17 runway Villavicencio - Puerto López. The use of animals was with prior written informed consent of the owners and approval by the Ethics Committee of the Universidad de los Llanos. For each bioassay, 9 mixed bovine animals were used with high to medium infestations of Rm (approximately 20 engorged females per animal), with average ages of 18 months and average live weight of 300 Kg; the bovines did not receive any kind of acaricide topical or systemic medication for a period of two months before the experiment; the animals grazed a grass meadow and had water at will; which were distributed in three experimental groups, where 3 animals underwent a spray bath with the solution of the extracts to the CL_{50} (data from in vitro analysis of each extract, not presented in this research, but published in 2); 3 animals were the negative control (water-Tween 80 2.5%); and finally 3 animals as positive control sprinkled with Amitraz 0.025%, for each experiment. The treatments in the bovines were carried out with manual back pump with pressure, each animal received treatment for ten minutes with 1 liter of solution for every 100 Kg of live weight.

For the evaluation of the effect of extracts in cattle, the methodology described by Bianchi et al. (3) was carried out, in which engorged females (ticks >4.5 y <8 mm body length) and larvae (ticks <4.5 mm body length) were counted on the right side of the bovine body, specifically in 4 areas of 10 cm^2: ventral lateral and dorsum lateral cervical aspect, tail base and perineum (Figure 1). Tick count was done before treatment (day 0), days 1 (D1), Day 2 (D2), and Day 3 post-treatment (D3). Daily tick counts were always at the same time (07:00-8 AM) and by the same researcher. At the end of the D3 the engorged females were collected moving them to *in-vitro* conditions, in this way, groups of 10 engorged females were organized for the different treatments, which were taken to incubation according to the AIT methodology for evaluating reproductive capacity after receiving the spray baths.

Results

Table 1. Mc effect against engorged females counts on the right side of bovine animals

Treatments	Day0	Day1	Day2	Day3	Average
Mc	9.33 ± 7.57^{A}	7.33 ± 5.51^{A}	6.67 ± 5.69^{A}	3.67±4,04	5.89 ± 1.95^{B}
Negative_Control	13.33 ± 9.02^{A}	9.33 ± 5.69^{A}	11.67 ± 7.51^{A}	NR	10.50 ± 1.64^{A}
Positive_Control	3.67 ± 2.31^{B}	2.67 ± 1.53^{B}	6.67 ± 4.93^{A}	NR	4.67 ± 2.82^{B}

Different letters in the same column indicate significant differences $p<0.05$, ANOVA posthoc Tukey–Kramer.

Conclusions

The results obtained from *in-situ* study developed validate the ethnopharmacologic reports of the use of the Gs and Mc plants as acaricides; in addition to linking as a new candidate to the plant Me as phytotherapeutic promising in animal production systems.

1826 - MYCOTOXICOSIS IN CATTLE AND BUFFALOES OF KARNATAKA AS CAUSE OF THE OBSCURE DISEASES : AN INNOVATIVE APPROACH FOR DIAGNOSIS AND THERAPY

Author: Dr N B Shridhar , S Yathiraj, Niranjan D

Objectives
A study was carried out in the state of Karnataka from 2002 to 2023 regarding the toxicity episodes caused by giving cattle feed ingredients contaminated with fungi. The toxicity resulting from feeding the fungal-infected sorghum fodder, ground nut hey, paddy straw, sorghum straw, maize hulls, medow grass, and commercial bovine feed was one of the mycotoxicosis episodes.
Material and methods
The animals, particularly the cattle, displayed a variety of clinical symptoms, including dyspnea, paraplegia, salivation, and faltering gait. Buffaloes were particularly prone to mycotoxicosis. From the suspected feed or fodder, the fungus were separated and identified. A symptamatic therapeutic regime was also established.
Results
Aspergillus niger, Rhizoctonia, Rhizopus, Macrophomina phaseolina, and Penicillium species from different grasses were the mycotoxins that were identified and found to be responsible. Rhizoctonia leguminicola, the predominant fungus in sorghum grass, has been known to be poisonous to mammals. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in blood serum were significantly ($P<0.05$) higher in sick cattle and buffaloes than in healthy cattle and buffaloes from other Karnataka regions. Haematological measures, including blood serum calcium, magnesium, bilirubin, blood urea nitrogen, and creatinine concentrations, as well as haemoglobin, total and differential leucocyte counts, were unaffected. Following a 5-day course of atropine (2 µg/kg i.m. twice a day), the clinically sick individuals healed. The reaction to atropine treatment implied that the cholinomimetic mycotoxin slaframine, which is generated by the predominant Rhizoctonia leguminicola fungus, might have had a role. The intravenous potassium acetate treatment, administered as a 2.5% solution at a dose of 10 mg/kg, was well received by the downer animals.
Conclusions
Mycotoxicosis is an important diseases of bovines and very less research is done in India and most of the time it goes undiagnosed or unnoticed. In the present study, the different obscure diseases were investigated and were diagnosed as mycotoxicosis, identified the fungi secreting the mycotoxins, different mycotoxins causing the toxicity in bovines and a therapeutic and preventive measures were suggested.
Key words: Mycotoxicosis, Cattle, Buffaloes, Karnataka, India, Obscure Diseases

1827 - ANAMIRTA COCCULUS TOXICITY IN MALNAD GIDDA CATTLE IN WESTERN GHAT AREAS: AN UNCOVERED OBSCURE DISEASE

Author: Dr N B Shridhar , Yathiraj S, D Niranjan

Objectives

Plant toxicity is a common cause of the morbidity and mortality of cattle and buffaloes in Western Ghats of Karnataka State, India. Malnad Gidda cattle are habitat of Western Districts of Karnataka State and let out for grazing in forest and do have more access to toxicity of plants. *Anamirta cocculus* is a climbing plant from India and other parts of Southeast Asia. It is is known for its large stems of white wood and scented flowers. During the fruiting season, Malnad Gidda cattle do consume the fruits fallen on the ground because of the sweet nature of the pulp of the fruits. The seed of fruits of the plant do contain a potent toxin picrotoxin , which is a very strong neurotoxin in all vertebrates affecting CNS. Fresh or dry semi-ripe fruits with or without the fruit pulp is ground and used as fish stupefying agent. In India and South-East Asia the fruit of *Anamirta cocculus* is used mainly as a fish poison

The data on toxicity features of *Anamirta cocculus* in cattle is scanty. Hence, the present study was aimed to evaluate the toxic feature of the plant and to study the changes in hematological and serum biochemical changes and also to evaluate the effectiveness of treatment.

Material and methods

In Chikkahodala village, Tirthahalli Taluk, Shivamogga District of Karnataka, 12 Malnad Gidda (8 Female and 4 males) cattle with age ranging from from 5-11 years reported to be exhibiting the clinical signs of anorexia, shivering, nervousness, lethargy and wobbling gait

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (CRT), calcium, magnesium, phosphorus and glucose were estimated in serum of the affected as well as the normal control Malnad Gidda animals. The Malnad Gidda cattle exhibiting the clinical signs of the toxicity were treated with diazepam @ 0.5 mg/kg slow IV, xylazine 0.1-0.2 mg/kg IM, B-complex vitamins, IM, intravenous administration of 5-10 ml/kg of 10 % dextrose solution, activated charcoal @ 2g/kg orally twice at 10 to 12 h interval for three consecutive days.

Postmortem of animals succumbed to death was conducted and the gross lesions were observed. Representative brain, heart, spleen, intestine, kidney, liver, rumen, and abomasum tissue samples were collected for histopathology at 10 % neutral buffered formalin (NBF).

Results

The clinical sign of *Anamirta cocculus* toxicity in Malnad Gidda cattle included clinical signs of, shivering, anorexia,nervousness, lethargy and wobbling gait. Most of the animals (7/11) succumbed to death indicated the severity of the toxicity.

Picrotoxin is the active principle of the fruit pulp of the plant *Anamirta cocculus*. Picrotoxin also acts as a convulsant. In larger doses, it has been found to induce clonic seizures or cardiac dysrhythmias, with especially high doses which was analyzed using LCMS.

In the present study, activated charcoal was administered at the dose of 2 g/kg orally as a 1:8 slurry which might have adsorbed the toxins from the gut.

In estimated biochemical parameters, an increase ($P < 0.05$) in blood glucose of the affected animals during the course of toxicity which returned to normal at 96 h with no change in ALT, AST, BUN,CRT, calcium, magnesium and phosphorus with an increase in in the blood glucouse. There was no change in the hematological parameters during the course of toxicity indicating that the toxicity had not affected system.

Administration of diazepam @ 0.25-0.5 mg/kg slow IV in an interval of 10-12 h and xylazine @ 0.1-0.2 mg/kg IM made satisfactory recovery of the treated animals subsiding the clinical signs and restoring their normal physical and physiological activity within 72 h.

The brain histological findings were degenerative changes in the neurons and glial cells, vacuolation and myelin degeneration with structural alterations in axosomatic and axodendritic synapses. Extensive hemorrhages in the brain of the affected Malnad Gidda animals was observed during the post mortem. There were degenerative changes in the neurons and glial cells.

Conclusions

Conclusion

A rare episode of *Anamirta cocculus* toxicity in Malnad Gidda cattle is studied here and put on the record and the different clinical signs etc with treatment were suggested.

Key Words: *Anamirta cocculus* Plant toxicity, Malnad Gidda cattle, Obscure Disease

GENETICS

1370 - Effect of the egg yolk of 5 avian species on the cryopreservation of ram semen

Author: Arturo Victor Gomez Gonzalez

Objectives

The study aimed to evaluate the effect on the cryopreservation of ram semen using egg yolk from five avian species. The experiment was carried out at the Ovine Genetic Improvement Center (CeMeGO), which belongs to the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of the State of Mexico.

Material and methods

Material and methods: To carry out this study, a clinically healthy Charollais ram was used, with a body condition of 3.5 (on a scale from 0 to 5) a weight of 105 kg and an age of 5 years. Semen samples were collected through an artificial vagina with a temperature of approximately 42º - 44º C. 3 ejaculates were obtained which were mixed in a single tube (pool), which was maintained in a water bath at 35ºC during the time in which processing was carried out. Once the semen sample (pool) was obtained, it was kept in a water bath at 35°C to determine its mass motility, individual motility and viability. Subsequently, it was diluted using a commercial diluent as a base diluent for the semen sample. The semen sample was divided into five aliquots containing 20% egg yolk from each of the five different avian species, that is, domestic chicken (Gallus gallus domesticus), Turkey (Meleagris gallopavo), Duck (Anatidae anas platyrhynchos), Quail (Coturnis coturnis) and Goose (Anser anser), diluted to a final concentration of 50 x 10^6 sperm/ml and frozen in 0.25 ml straws. Frozen semen straws were thawed by immersion in a water bath at 37°C for 60 seconds. Five straws of each of the different egg yolks were thawed and placed in individual cryovials that were kept in a water bath at 37°C for 0, 30, 60, 90 and 120 min of incubation and evaluated independently. The chicken egg yolk was considered as the control group. The results of the present study were analyzed through a repeated measurements design.

Results

Result: There were statistically significant differences ($P<0.05$) in mass motility, individual motility and viability of ram semen thawed and kept in incubation for 120 minutes in favor of quail, turkey and goose egg yolks concerning egg yolks. duck and chicken.

Conclusions

Conclusions: Under the conditions in which the present experiment was carried out and based on the results, it is concluded that quail, goose and turkey egg yolk provided the best cryoprotective action to ram sperm during the freezing-thawing process in terms of motility. mass, individual motility and viability, which is why it is recommended that they be used as substitutes for chicken egg yolk in semen diluters for rams

1425 - Parenteral mineral supplementation, vaccine challenge and interleukin-6 and 4 response in crossbred dairy cows

Author: Natália Martins Barbosa, Luc Durel, Marcelo Siqueira El Azzi, Laura Souza Santiago, Ludmila Carvalho de Andrade, Lara Matias Lima de Oliveira, Jose Camisão de Sousa, Bruno Lima

Objectives

The objective was to evaluate the ability of parenteral supra-nutritional mineral supplementation to increase mineral concentrations in the blood and modulate the production of interleukins 6 (IL-6) and 4 (IL-4) in dairy cows, whether or not subject to a vaccination challenge. Across species, IL-6 increases indicate enhancemt of the immune response and the blocking of IL-4 improves the efficacy of vaccines in mice. The modulation of these cytokines through vaccination is of interest in dairy cows in order to improve the immune response to disease and promote general health and productivity.

Material and methods

Sixty Holstein/Gyr crossbred lactating cows were used, between 10 and 50 days postpartum, including primiparous and multiparous animals, with an average weight of 600 (±80) kg and average daily milk production of 32.0±5.3kg. The animals were housed in a compost barn, receiving diets (total mixed ration, including premix containing minerals) that meet their nutritional needs, as per the recommendations of the NRC (2021), in addition to free access to water. In a 4X4 factorial design, cows were blocked by parity and milk production, and randomly allocated to one of four groups with 15 animals each: Control (GC), Vaccine (GV), Mineral (GM), Vaccine and Mineral (GVM). Cows in the GVM group received two intramuscular injections of 10 mL of mineral supplement (FOSFOSAL®, Virbac, France) and the vaccine (BOVIGEN® Repro TOTAL SE Virbac, France) against IBR, BVD, leptospirosis and campylobacteriosis, with an interval of 30 days (D0 and D30). The GV animals received injections of 10 mL saline, intramuscularly (SA), concomitantly with the same vaccination protocol as the GVM. The GM cows received two intramuscular injections of 10 mL of mineral supplement on D0 and D30, while the CG received two injections of 10 mL of SA on the same days (D0 and D30). Four blood samples were collected from all animals on D0, D30, D37 and D44 of the experiment for IL-4 and IL-6 analyses, in addition to evaluating the body condition score (BCS) on the same days. D0 and D37 blood samples also served to analyze the concentrations of phosphorus (P), magnesium (Mg), potassium (K), selenium (Se) and copper (Cu). Data were analyzed using the JMP Pro-12 statistical package (SAS®, Cary NC, USA).

Results

The BCS increased (P=0.04) from 3.10 to 3.34 from D0 to D37. There was an interactive effect (P<0.05) on mineral concentrations between treatment and day of blood collection, considering only two groups (n=30) in this analysis: supplemented (GSs: GVM and GM) or not (GNSs: GV and GC). The serum concentrations of Cu and P increased in the GSs groups and decreased in the GNSs groups from D0 to D37 (P=0.02 and P=0.01, respectively). Se increased in GNSs and remained similar in GNSs between D0 and D37 (P=0.01) as did Mg concentrations in GSs, but decreased in GNSs (P<0.01). K was higher on D37 in GSs compared to GNSs (P<0.01). There was an interactive effect between

treatment and day (P=0.02) for IL-6 and -4 concentrations. IL-6 concentrations were higher (P=0.0002) in GVM and GM on D44, but similar to those in the non-supplemented groups on D0. There was an interactive effect (P=0.05) between treatment and collection day for IL-4, with its concentrations being similar among all groups on D0, but lower in the supplemented groups compared to the non-supplemented groups on D44. Furthermore, IL-4 concentrations did not differ between the supplemented groups.

Conclusions

In conclusion, under the conditions of the present experiment, parenteral supra-nutritional supplementation provided higher blood concentrations of the minerals evaluated in the postpartum period compared to no supplementation. Parenteral supplementation was able to modulate interleukins 6 and 4 in non-vaccination (GM group) and post-vaccination (GVM group) conditions, with the increase for IL-6 being more exacerbated after vaccination in the supplemented group compared to the others. This modulation clearly indicates that the supra-nutritional supplementation of minerals essential to the physiological functions, of lactating dairy cows, can activate the immune system, producing an environment more conducive to combating infectious diseases and a consequent increase in production efficiency, reproductive health and the sustainability of dairy cattle farming.

1558 - Novel Bovine Reference Gene Candidates for RT-qPCR Identified via Search of Publicly Available Tissue RNA-seq Datasets

Author: Cassandra Barber , Matthew Scott, Amelia Woolums

Objectives
Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) is commonly used to characterize gene expression for various research and diagnostic applications. However, relative quantitation of gene expression requires the use of reference genes to normalize differential gene expression. Current recommendations for best practices in RT-qPCR indicate that some previously used reference genes lack stable expression under certain experimental conditions. Thus, there is a need to identify new reference genes with stable expression across different tissues and experimental conditions. While there are a plethora of new studies identifying stable reference genes for humans, mice, and rats, there are few studies confirming stably expressed bovine reference genes. Next-generation RNA sequencing, or RNA-seq, can be used to identify the entire repertoire of genes expressed in a tissue. Transcriptomes generated by RNA-seq allow the identification of stably expressed reference genes. The objective of this study was to identify candidate bovine reference genes using publicly available transcriptomes.
Material and methods
Forty-one transcriptomes were acquired from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO). Datasets were acquired from projects having sampled various organ systems in apparently healthy *Bos taurus* cattle; data from 11 different tissue samples were accessed, including bone marrow, bronchial lymph node, mesenteric lymph node, kidney, liver, lung, nasal epithelium, nasopharyngeal lymph node, spleen, thymus, and trachea. Raw sequenced read files for each dataset were quality assessed with FastQC v0.11.9 and trimmed with Trimmomatic v0.39. Trimmed read fragments were mapped to the bovine reference assembly ARS-UCD2.0 via STAR v2.7.11a. Raw gene counts generated for each transcriptome dataset were processed and analyzed in R v4.0.2. To adjust raw gene counts for uncontrolled variance, or batch effects, across each BioProject submission, the “ComBat_seq” empirical Bayesian function from the Bioconductor package sva v3.46.0 was utilized. Library normalization was performed with the Relative Log Expression (RLE) method, using default settings. The NormFinder algorithm was then applied to the complete dataset, retaining 2000 candidate reference genes ranked by dataset expression stability values.
Results
The top twenty identified candidate reference genes are as follows with the respective stability value: CRNKL1 (0.175), SPOP (0.202), NMT1 (0.216), PIGU (0.217), YKT6 (0.232), GZF1 (0.251), ARIH2 (0.257), MTERF4 (0.272), CERS5 (0.277), SNUPN (0.280), RANBP9 (0.281), C11H9orf78 (0.281), POP4 (0.284), TMEM183A (0.286), DDX23 (0.288), UBE2Q1(0.291), GNL2 (0.292), UBAP1 (0.294), FBXW11 (0.300), and TTC4 (0.300).

Conclusions
Notably, these candidate reference genes are stably expressed genes across all the tissue types based on RNA-seq experimentation. This analysis of the transcriptomes

serves as a foundation for RT-qPCR analysis and the genes identified as candidate reference genes will be validated for stability in future research.

1589 - A case report of congenital hypotrichosis and hypodontia in a Holstein Heifer

Author: Juliana Melo Pankratz, Rüdiger Daniel Ollhoff , Ana Carolina Martins Bock, Diógenes Adriano Duarte Santana

Objectives

Report a clinical case of hypotrichosis in a Holstein calf and its further development until the age of 20 months at the experimental farm hospital of Gralha Azul, PUCPR, located in the municipality of Fazenda Rio Grande, state of Paraná, southern Brazil.

Material and methods

A female Holstein calf at the age of 6 months was donated by a dairy farm to the hospital, due to hypotrichosis and lack to withstand solar exposure. The animal was maintained for educational purpose until the age of 20 months. Even small amounts of insulation during cloudy days caused sunburns, so the heifer was permanently stabled, reaching 665 Kg. The body condition score was 3 (1-caquetic to 5- obese). During her stay at the hospital, the temperature, respiratory, cardiac, ruminal parameter always stayed in normal range. Overall the presented hair seemed less dense, a bit curlier and in specific areas there were patches of alopecia, such as near the eyes, nostrils, scapulohumeral joint and insertion of the tail. The specific examination of the oral cavity revealed in the left superior and inferior hemiarcade {I 0/4 P 3/2 M 3/3} and the hemiarcade on the right side {I 0/4 P 3/2 M 2/3}. The deciduous incisors were worn out but at the age of 20 months still not erupted. The measures of the head were uneven and showed a slight asymmetry with the right side (47cm) measured from the nasolacrimal duct until the nose cavity shorter than the left side (48 cm). An X-ray examination was realized in the dorsoventral region of the mandibular body confirming hypodontia and the presence of the permanent incisors not yet erupted. Tissue for genetic examination was sent abroad (University of Bern, Switzerland) but results are still not available. Attempts were made to attenuate sunburn with homemade sunblockers used topically.

Results

This is to our best knowledge the first case of this kind of hypothrichosis in Brasil surviving as long as 20 months. As even stabled dairy cattle are eventually exposed in Brazil to sunlight a heifer with this kind of anomaly could not survive maintaining minimal welfare aspects and skin health under normal production conditions. The experimental use of a homemade sunblocker was no solution, as the animal cleaned himself. Dental anomalies were reported associated to other cases of hypotrichosis in the international literature.

Conclusions

Under controlled conditions, without exposure to sunlight, even cattle with hypodontia could reach a good weight with good body condition. Hypotrichosis associated to hypodontia are rare clinical conditions seldomly reported in the literature and to our knowledge with the survival up to 20 months not reported in Brazil. As the maintenance of a dairy heifer under this condition is expensive, most of the farmers probably would cull the animal.

1597 - Genetic-molecular characterization of Creole goats in the Mountain of Guerrero, México

Author: Irlanda Judith Siliceo Cantero, Ricardo Lobato-Ortiz, Martha Hernández-Rodriguez, Glafiro Torres-Hernández, Samuel Vargas-López, Javier Suarez-Espinosa, Omar Hernández-Mendo

Objectives
The objective of this study was to obtain information about the genetic reservoir that is housed in Criollo goats in Mexico.

Material and methods
Samples were taken from a population located in Tlalixtaquilla, Guerrero, with coordinates 17° 20' 25" and 17° 42' 29" north latitude and 98° 26' 48" and 98° 48' 37" west longitude, managed in an extensive production system with night confinement. Feeding is based on grazing for approximately 8 hours a day with a displacement of 2 km to the areas where plant species such as kickstand, tehuixtle, hawthorn, among others, are found(García Bonilla et al., 2018). Jugular vein whole blood samples (approximately 5 mL) were collected from 70 non-pregnant goat females between 3 and 5 years of age, which were kept refrigerated and then frozen until use. Subsequently, DNA extraction was done with the Wizard® Genomic DNA Purification Kit, no. catalogue A1120 of the Promega® brand. Ten microsatellites recommended by the ISAG/FAO committee for the analysis of the genetic diversity of domestic animals(FAO, 2004) were amplified which was done by PCR (polymerase chain reaction), then SDS-PAGE was made of each amplified sample and finally the resulting gels were analyzed with the GelAnalyzer ®23.1 software, with which the information was obtained to make a database to be later analyzed with the Power Marker® software, to obtain the following variables: number of alleles per marker, effective number of alleles, observed and expected heterozygosity, and polymorphic information content. (Silva-Jarquin, 2018)

Results
The results showed a great genetic diversity in this population. The markers were very informative because of their polymorphism.

Conclusions
The information obtained in this study is important because it allows us to know the genetic diversity of the goats of Mexico, thus being able to design genetic improvement programs with greater knowledge and better targeted. In addition, it is used to develop conservation programs for this genetic material.

1608 - Genome-wide scan for selection signatures in Tropical Milking Criollo cattle

Author: Ricardo Emmanuel Martinez Rocha, Hugo Oswaldo Toledo Alvarado, Rosa Higuera Piedrahita

Objectives

Tropical Milking Criollo (TMC) is a tropically adapted breed developed due to the geographic isolation of the Criollo cattle and farmers' selection of milk production traits. We aimed to identify and characterize selection signatures in TMC using medium-density SNP data by two approaches: 1) Runs of homozygosity (ROH) and 2) the Wright's Fixation index (F_{ST}).

Material and methods

Samples of 50 TMC animals and 50 Holstein cows were genotyped with 100k SNPs. All considered TMC animals belonged to the *Asociación Mexicana de Criadores de Ganado Romosinuano y Lechero Tropical*. Quality controls on animals and markers of the two samples were performed according to the following parameters: minimum SNP call rate of 90%, minor allele frequencies < 0.0005, only autosomal SNPs were considered, and only individuals with high genotyping (at least 90% complete call rate) were included. The genotype data of TMC was used to obtain ROH segments considered regions under selection. The F_{ST} values were estimated by comparing the TMC with the Holstein animals.

Results

Only one region mapped to 38.57-39.22 Mb on BTA20 was considered a selection signature by the ROH method. This selection signature overlapped with a diversification region between TMC and Holstein breed by F_{ST}. The SPEF2, PRLR, AGXT2, TRNAY-AUA, DNAJC21, and BRIX1 genes were found in that ROH island. The PRLR gene has been related to the Slick hair phenotype, also found in other criollo cattle breeds. Using the F_{ST} approach it was also found that the MC1R gene diverged in TMC compared to Holstein cattle. The MC1R gene has been associated with coat pigmentation.

Conclusions

The selective signals identified in this study reflected the direction of the selection pressure that primarily involves adaptative traits to tropical climates in the TCM cattle.

1609 - Genetic diversity of SNPs associated with diseases in dairy and dual-purpose cattle in Veracruz, México

Author: Vicente Eliezer Vega Murillo, Maria Luisa Méndez Ojeda, Raymundo Salvador Gudiño Escandón

Objectives

Several studies have demonstrated the utility of SNP dense genotypes to identify chromosomal regions associated with autosomal recessive conditions in cattle (Charlier et al., 2008; Seichter et al., 2011 and Michelizzi et al., 2001). VanRaden et al. (2011), presented the discovery of five haplotypes with deleterious effects on fertility in three dairy cattle breeds, including one recessive in Brown Swiss cattle, three in Holsteins and one in Jerseys. The objective of this study was to determine their genetic diversity in two different production systems, 25 single nucleotide polymorphisms (SNPs) previously associated with disease-related traits in specialized and dual-purpose dairy systems in the central zone of the state of Veracruz were used.

Material and methods

The genotype of 290 cattle from milk production herds in the high lands (located in the Municipality of La Joya, purebred Holsteins) and dual-purpose herds in the lowlands (located in the Municipality of Medellin, Holstein x Zebu crosses), of the state of Veracruz, were used with the BovineHD Genotyping Beadchip panel containing 150,000 SNPs. Allele frequencies, genotypic frequencies, observed (Ho) and expected (He) heterozygosity, Wright's fixation index (FST) and deviation from Hardy Weinberg equilibrium (EHW) were calculated. Of the total number of animals and markers.

Results

: 25 markers and 278 animals passed the quality control. The genotyping rate of the animal sample was 0.99. All SNPs evaluated were polymorphic. For most markers, in both populations, Ho and He values were similar, so the EHW was not significant ($p > .05$). only in two SNPs (BTA-107511-no-rs and BTB-00466773) of the whole population studied, the observed and expected heterozygosity values were different ($p<0.05$). The frequencies of the major allele ranged from 0.53 to 0.91 with an average of 0.71. The markers associated with laminitis problems (BTB-00466773, BTA-107511-no-rs2, and Hapmap50590-BTA-120045), clinical mastitis (BTB-00803496, BTA-28763-no-rs and BTA-36568-no-rs) and fertility (ARS-BFGL-NGS-10627) had frequencies of the major allele ≥ 0.80. For most markers, in both populations, the values of Ho and He values were similar, so that the EHW was not significant ($p > .05$). ($p > .05$). Only in two SNPs (BTA-107511-no-rs and BTB-00466773) out of the entire population studied, the observed and observed and expected heterozygosity values were different ($p < .05$). For the dual-purpose cattle population, the minimum, maximum and average allele frequencies for the major allele (p) among the markers studied were 0.42, 0.92 and 0.67, respectively. For the intensive cattle population, the minimum, maximum and average allele frequencies for the major allele among the markers studied were 0.51, 0.94 and 0.74, respectively. The markers with the highest minor allele frequency in the dual-purpose population were: Hapmap41584-BTA-56031, Hapmap59332-rs29016542, Hapmap60335-rs29018229 and BTA-55821-no-rs with frequencies between 0.58 and 0.50. For the population under the intensive system, the markers with the highest frequencies of the minor allele were markers with the highest frequency of the minor

allele were BTA-47853-no-rs and BTA-27247-no-rs for the population under the intensive system. The average Wright's fixation coefficient for this population was 007 with a minimum of 0.00 and a maximum of 0.00 and 0.00 respectively. of 0.00 and a maximum of 0.28. The markers where the most differentiation was found were: BTA-107511-no-rs, ARS-BFGL-NGS-114992, BTA-55821-no-rs and Hapmap60335rs29018229 with values greater than 0.14.

Conclusions

Specialized and dual-purpose dairy cattle populations in the central zone of the state of Veracruz, México show high frequencies of some markers of interest and allowed us to know the genetic structure for SNP-type markers that have been previously associated with candidate genes for disease. The frequency of the markers associated with candidate genes for some diseases in cattle does not necessarily imply the presence of these diseases in cattle populations due to the polygenic nature of the nature of these traits.

1629 - A rare case of polymelia in a fattening calf

Author: Aleksander Butkiewicz, Bartłomiej M. Jaśkowski, Grzegorz Balicki, Maciej Zdun, Kacper Żukowski, Jędrzej M. Jaśkowski

Objectives

Developmental abnormalities in cattle attract the attention of breeders and veterinarians for many reasons. Most of these abnormalities arise in the prenatal period; sometimes, they are also observed in postnatal life. They are then usually the result of malnutrition or the use of drugs with chondrotoxic effects. Developmental abnormalities can have their origin in hormonal disorders, genetic defects, metabolic disorders, and infectious diseases.

Polymelia is a congenital and inherited malformation that manifests itself in the presence of extra limbs. This rare defect has been observed in many breeds of cattle, both beef and dairy traits. However, nowadays, given the possibilities of veterinary medicine and the economic aspects, the disease is mainly a research area. From the perspective of the general spectrum of developmental defects in cattle, hyperplasia of the limbs appears to be one of the simpler clinical cases. Appropriate surgical intervention can result in a complete cure by removing redundant limbs, but this is often not mandatory.

This summary describes the case of a Holstein-Friesian calf that showed *polymelia* in the withers region. Diagnostic tests and dissection of the supernumerary limb were performed.

Material and methods

A calf was reported in August 2023. The patient was a male Red Holstein-Friesian calf with an unusual lesion around the withers. According to the interview, it was born on January 27, 2023. It came to the farm at the age of 3 weeks for fattening. Initially, the strange change was the size of a small coin. The lesion grew rapidly in proportion to the calf's development.

Results

Under the skin of the lesion, a fragment of a miniature limb could be felt, attached to the shoulder blade on one side and ending with small claws from the outside. Palpation examination revealed that the calf had difficulties with moving its head to the left. An X-ray performed under field conditions confirmed the extra limb's permanent fusion with the individual's skeletal elements. The X-ray showed the absence of hard tissues inside the deformed humerus. However, the phalanges were visible, which do not produce a joint together with the metacarpal bone. The calf was slaughtered at the age of 7 months. The lesion was submitted for further pathological examination.

Conclusions

The incidence of *polymelia* is estimated at 1 per 100,000 cattle births. Such defects can lead to economic losses on livestock farms, reducing the value of the animals and negatively affecting their growth. Slaughter of such animals often becomes the only option. Surgical treatment of *polymelia*, although it exists, is difficult to carry out for economic reasons.

To the best of our knowledge, this is the first description of a *polymelia* of a Holstein-Friesian red-white calf. In the next steps, genetic studies are planned in the context of further case diagnostics and the identification of genetic causes of *polymelia* (gene

mutations) in the Holstein-Friesian breed. This research appears to be important in that it may contribute to the earlier detection of animals with this defect and, consequently, the screening of developmental defects in the cattle population through the placement of suitable probes on SNP microarrays.

1697 - Genetic trends for longevity using a linear mixed model in the Mexican Holstein cattle population

Author: Sandra Giovanna Nuñez Soto, Adriana García Ruiz, Hugo Oswaldo Toledo Alvarado, Felipe de Jesús Ruiz López

Objectives

For more than a decade, international genetic evaluations have included longevity as an economically important trait for dairy cattle, this trait reduces the replacement costs per cow in the herd and increases average yield by increasing the proportion of cows in the higher producing age groups and increasing the possibilities for voluntary culling (Vollema and Groen, 1996). Longevity has been shown to exhibit genetic variability, generally low but sufficient for genetic progress in subsequent generations. In the USA, genetic trends have been obtained for longevity measured as herd productive life where they demonstrate over a period of almost 40 years the positive impact that including this trait in the country's selection program has had (García-Ruiz et al.,2016). In Mexico, the evaluation of longevity in Holstein cattle is performed with a sire model of survival (Abadía et al., 2016) that although it efficiently incorporates partial information of cows still alive at the time of evaluation, it has the limitation that it only calculates direct values for sires, so using linear models based on a measurement such as MEP84 that presents advantages because it is a continuous variable that better represents the useful life of a cow, makes the distribution of the variable closer to the normal distribution and allows for both complete data up to culling for very old cows and censored data for younger cows (Van Raden and Klaaskate, 1993) could be very useful. The objective of this study was to obtain the predicted genetic values (EBV) and their genetic trend over a 24-year period to observe the impact of including this variable in the genetic evaluation of the Mexican Holstein population.

Material and methods

Information from the production control system and herd registry of the Holstein Association of Mexico was used. A total of 70,313 complete and censored records of cows with 1 to 5 calvings, information on milk production in kg adjusted to 305 days (PE), cumulative months in milk (MACL), months in production at current calving (MLPA), gestation index (IDG) (0= not pregnant, 1= pregnant), lactation status index (EDL) (0= milking, 1= dry) and age at first calving in months were included. Longevity was measured as months in production at 84 months of age (MEP84) which was calculated by setting a limit of 10 months in milk production in each lactation according to the methodology of VanRaden and Klaaskate (1993). A linear model was fitted to predict longevity measurements from the productive variables and age at first calving in its linear and quadratic effect. The fixed effect of herd-year at first calving was included. The GLM procedure of SAS statistical software was used. After the predicted MEP84 values were obtained, an expansion factor was applied according Van Raden and Klaaskate (1993) in order to homologize the genetic variances of complete and censored data and to include them in the same genetic analysis. The predicted genetic values were obtained with an animal model including the herd-year-first calving season fixed effect, the sire x herd random effect and the animal random effect using the AIREML program of the BLUPF90 software.

Results

Genetic trends were obtained from a genetic base of animals born in 2015. It is observed in females born in a period from 1996 to 2020 that the trend of EBVs is increasing -4 to 2 months in production. On the other hand, in males born from 1996 to 2012, an increase is observed, but from the year 2013 to 2020, the trend is observed to be decreasing, similar results were reported in the study of Abadía et al., 2016. One cause of this trend in males could be that farmers are establishing milk production as their main selection criterion and this may cause infertility problems and other problems related to health and conformation, which may cause farmers to increase involuntary culling in this population of cows.

Conclusions

In general, genetic trends have been increasing, indicating a rapid genetic improvement in longevity in the evaluation of this population. It is necessary to include MEP84 in the genetic evaluation, because it represents an objective and easy to understand measure for breeders and because it will improve the voluntary culling of this population, which would improve the selection programs of this population.

1736 - GENETIC EVALUATION OF RUMINATION TIME OF DAIRY CATTLE LOCATED IN CENTRAL-WESTERN MEXICO

Author: Felipe de Jesús Ruiz-López , Areli Daniela González Sandoval, Adriana García-Ruiz, Hugo Oswaldo Toledo-Alvarado, José Luis Romano-Muñoz

Objectives

To estimate variance components, heritability (h^2), repeatability (t) and genetic correlations (r) for milk yield (MY, kg/day), rumination time (RT, min/day), and daily activity (DA, min/day) using repeatability animal model (RAM) and estimation of predicted genetic values for each trait.

Material and methods

The study included information collected from 7,370 lactations in 5,135 cows, corresponding to the years 2019 to 2023 from commercial herds located in central-western regions of Mexico. Each herd was managed independently in terms of feeding and different diet formulations. The process of the data and estimation of descriptive statistics was performed using SAS (version 9.4). Available information was collected through the Allflex DataFlow™ II system, and measurements of TR and AD from the SCR Heatime® HR System (Copyright© 2015 SCR Engineers Ltd). RT, DA and MY records were summarized by herd and parity number in which the cow was found. The identification and elimination of extreme data was carried out using the range of 3 standard deviations (σ) from the mean, as well as a filtering of unrealistic values or values considered as an error in the information record, for each trait by herd. Records of cows with missing data on RT or MY were not allowed. Finally, contemporary groups were defined by combining the information of the herd, year, and season (HYS) according to the date of data recording. Contemporary groups with less than 5 animals were merged with adjacent groups.

The (co)variance components, phenotypic correlations, genetic correlations, and genetic values were estimated using Restricted Maximum Likelihood and the BLUPF90 software with univariate and bivariate RAM for RT, DA and MY. The models used in this study included an additive genetic, a permanent and a residual effect as random and the calving number (CN), MY, and HYS as fixed effects for RT and DA. The model for MY was similar, but only had CN and HYS as fixed effects.

Results

The estimated descriptive statistics (μ±σ) of the analyzed traits were 543.4±41.5 min/day, 513.1 ±63.3 min/day, and 36.4±14.5 kg/day for RT, DA and MY, respectively. Similar values had been reported in the literature (Andreen et al., 2021): RT (μ ± σ) 527 ± 73 min/day and a MY of 38.3 ± 10.3 kg/day; Reith et al. (2014): RT values (μ ± standard error) of 445.8±17.8, 426.7±16.6, and 407.3±16.5 in cows with MY of <35, 35-40, and >40 kg/day, respectively).

The **h^2** (h^2±σ) estimated for RT in our population was high 0.40±0.054 similar to those estimated by Moretti et al. (2017), (0.31±0.05 to 0.36 ±0.05), Byskov et al. (2017) (0.33±0.16) and Lopes et al. (2023) (0.41±0.15). The estimation of **t** (t±σ) for RT was 0.51±0.01, which was lower than the range reported by Byskov et al. (2017) from 0.75 to 0.80. The estimated genetic correlation between RT and MY was positive (0.15±0.05), implying that selection made on MY will increase RT in future generations. The **h^2** (h^2±σ) for MY was 0.25±0.004, and similar values had been reported in the

literature for Holstein dairy cattle from México ranging from 0.15-0.20 (Martinez et al., 2020) to 0.16-0.5 (Toledo et al., 2014).

The average genetic correlation between RT and DA was –0.16 ±0.16, possibly because the time allotted to rumination has to be taken away from the daily activity of the animal. Estimated $\mathbf{h^2}$ and **t** for DA in our population was 0.10±0.01 and 0.53± 0.01, respectively. We did not find reported values for this genetic parameter for DA.

Conclusions

RT had a moderate heritability, highly repeatable and positively associated with MY. Selection for increased RT will increase MY, but its use in selection programs requires more investigation as well as a larger dataset.

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1750 - HAPLOTYPES RELATED TO MILK PRODUCTION AND ITS COMPONENTS IN HOLSTEIN CATTLE FROM MEXICO

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Objectives

To identify haplotypes (HAP) associated with milk yield (MY), its fat content in kg and percentage (FY, FP), protein in kg and percentage (PY, PP) and somatic cell score (SCS) with genome wide association studies (GWAS) in Holstein cattle.

Material and methods

The analysis included 640,746 lactation records adjusted to 305 days for MY, FY, FP, PY, PP and SCS. Data from from 1 to 3 freshening's of 358,857 Holstein cows.

The genomic information included 9,095 genotypes, with 106,318 imputed single nucleotide polymorphisms (SNP), which were previously filtered by quality control, which consisted of excluding animals with call rates < 0.95, or with conflict of parent´s < 1%, and SNP with minor allele frequency (MAF) < 0.05, or with call rate < 0.95, or P value for the Hardy Weinberg equilibrium test < 0.15.

The construction of HAP was carried out with the PLINK 1.07 software with the --blocks parameter, with a minimum distance of 200 Kb between SNP, a linkage disequilibrium (LD) of r^2 > 0.80 and frequency > 1% in the population. The HAP were recoded as pseudoSNP into 2, 1 and 0 corresponding to the presence of two copies, one copy or the absence of paternal and maternal alleles for each HAP, obtaining a total of 35,552 pseudoSNP, excluding the X chromosome (CHR) and applying the control quality.

The pseudoSNP were used for the GWAS of the 6 traits, carried out with the BLUPF90 programs that is based on the prediction of the genetic values (GV) by ssGBLUP and the posterior estimation of the effects of the HAP to carry out the association between HAP with the traits by ssGWAS.

The model used to predict the GV for the traits was:

$$yijklmn=\mu+HYSi+AGEj+PEk+SHl+Am+eijklmn$$

Where *yijklmn* is an observation (phenotype) for each of the six analyzed traits and fixed effects were: μ as the overall mean; *HYSi* the i-th herd-year-season level and *AGEj* the j-th age level at calving within lactation. Random effects included were: *PEk* which is the k-th level of the permanent environment; *SHl* as the l-th level of the sire-herd combination: *Am* representing the animal effect, and *eijklmn* the residual effect.

Results

In this study 11,788 HAP were found with an SNP average of 3.48 ± 2.44, a minimum of 2 and a maximum of 59, the average length was 41.06 Kb with a minimum of 0.003 and a maximum of 199 Kb.

HAP associated with SCS were not found. For the other traits, 77 HAP had a significant association (–log10(0.01/35,552) = 6.55) and were found on CHR 3, 5, 6, 14 and 20. Of all the associated HAP, 23 were exclusive to PP, 16 for FP and highlighted 2 HAP of 5 SNP each in CHR 14 that were associated with all the traits in the region from 1.51 to 1.69 Mb and 1.80 to 1.92 Mb. In the first region, SNP associated with the PPP1R16A and FOXH1 genes were identified. In the second region, associations with the DGAT1 gene with effects on MY and FY were reported. In CHR 5, a HAP associated with FY and FP stood out; in this region (93.5 to 95.6 Mb) the association with the ITPR2 gene with

effects on MY and FY was reported. In the region of 38 to 38.2 Mb of CHR 6 where a HAP of 3 SNP associated with PP stood out, SNP associated with the ABCG2 gene with effects on MY were reported. In CHR 20 in the region from 31.9 to 32.1 Mb, a HAP with 9 SNP associated with PP was found, in this region SNP associated with the GHR gene were showed.

In most HAP that showed association, SNP associated with the productive traits of milk and its components have been reported, which could show selective processes in the analyzed population.

Conclusions

The significant HAP in this study are related to the presence of genes previously reported with effects on productive traits which shows the presence of genomic regions with high LD and stability in Holstein cattle from Mexico.

1762 - EFFECTS OF HOMOZYGOUS REGIONS ON MILK FAT AND PROTEIN YIELD IN HOLSTEIN CATTLE

Author: Adriana García Ruiz, José Cortés-Hernández, Felipe de Jesús Ruiz López, Francisco Peñagaricano, Hugo H Montaldo

Objectives

To quantify the effects of homozygous regions (HR) on milk fat yield (FY) and protein yield (PY) in Holstein cattle.

Material and methods

The phenotypic information included 204,288 productive records, previously adjusted to 305 days and mature equivalent, belonging to 106,547 cows of the Holstein breed, which had FY and PY information for up to 3 lactations. The animals were distributed in 353 herds in 18 states of the Mexican Republic. The genomic information consisted of 7,713 cow genotypes which had information on 88,911 SNP type markers previously imputed with FindHap V2 and filtered by the genomic quality control, which consisted of excluding animals with a call rate < 0.95, or with a parent's conflict < 1%, and SNP with minor allele frequency (MAF) < 0.05, or with a call rate < 0.95, or with a P value for Hardy Weinberg equilibrium test < 0.15 or monomorphic. Quality control was performed using the BLUPF90 family of programs.

The identification of the HR was carried out using the PLINK 1.9 software that defines them as homozygosity runs, using the –homozyg function and the inclusion of the following parameters: minimum HR window length of 20 SNP, with a maximum distance between SNP of 1000 Kb, no heterozygous SNP per run and 1 missing genotype allowed. To calculate the effects of HR on productive traits, those present in more than 200 animals. The analysis used a mixed linear model for the prediction of genetic values (GV) for FY and PY, using the BLUPF90 family of programs. Significant effects were assessed using the t-test (|t value| ≥2).

The model used to obtain the effects was the following;

$$y_{ijklmno}=\mu+HYS_i+AGE_j+PE_k+SH_l+{}^{1}\sum_n HR_m+A_n+e_{ijklmno}$$

Where $y_{ijklmno}$ is an observation (phenotype) for each of the analyzed traits and fixed effects were: μ as the overall mean; HYS_i the i-th herd-year-season level and AGE_j the j-th age level at calving within lactation. Random effects included were: PE_k which is the k-th level of the permanent environment; SH_l as the l-th level of the sire-herd combination: ${}^{1}\sum_n HR_m$ is the effect of each HR considering its presence or absence in the animal: A_n representing the animal effect, and *eijklmno* the residual effect.

Results

The adjusted average production at 305 days for FY was 393.80 ± 96.67 and for PY was 363.30 ± 81.81 kg.

In the studied population, a total of 376,364 HR were found, with an average of 33.19 ± 3.17 HR per animal and an average length of the HR was 6,910.82 ± 1,420.70 Kb. 8 HR present in more than 200 animals were detected on chromosomes (CHR) 2, 10, 15, 16, 17, 19 and 20, but only 4 had significant effects (p<0.01) on FY and PY. All the effects found resulted in a decrease in productive performance of the evaluated traits, in CHR 2 one HR (45.9 - 48.5 Mb) with 109 SNP affects the decrease in PY by 9.64 ± 3.89 Kg, in CHR 16 the HR (4.4 - 52.2 Mb) with 214 SNP showed a decrease of 13.73 ± 4.86 and 11.14 ± 4.04 Kg for FY and PY. Another RH in CHR 17 (0.05 – 2.65 Mb) affects PY by 9.28

± 3.87 Kg and in CHR 20 the RH (34.9 – 39.1 Mb with 156 SNP) presented the highest effect, decreasing up to 31.61 ± 16.07 for FY in a lactation.

The correlation found between the effects for the GV of FY and PY was r = 0.87 ($p<0.01$), which indicates that the effects of HR decrease the productive performance of both traits.

In the HR regions, several associations of SNP and QTL (QTLdatabase) related to the traits evaluated and others such as milk production have been reported, although these regions show effects in the decrease in production, they show the objectives of selection that have been carried out in the analyzed population.

Conclusions

HR showed negative effects on FY and PY production in Holstein cattle from Mexico. The presence of some HR in the population, probably caused by inbreeding, leads to a decrease in milk components such as fat and protein.

1800 - Evaluation of growth and different measures of efficiency in Red Brangus cattle in Mexico

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Objectives

The purpose of genetic improvement in beef production is to identify superior individuals for economically important traits and crossbreeding schemes that allow for efficient meat production. Meat production in Mexico, as it is currently developed, is not efficient. It is necessary that the tools available for genetic improvement continue in the medium and long term with the identification of animals that contribute to increase the efficiency of meat production and, especially, to make cow-calf production an economically profitable activity (Ríos, 2023). The objective of this work was to estimate environmental factors affecting growth up to one year of age and measures of growth efficiency in Red Brangus cattle in Mexico.

Material and methods

Production records of 31,768 Red Brangus calves born between 1995 and 2022 belonging to breeders of the association of Red Brangus of México A.C. were used. The variables studied were birth weight, weaning weight adjusted to 205 days, yearling weight adjusted to 365 days, The Kleiber index was calculated by dividing the average daily weight gain (ADG) by the metabolic live weight (0.75) at weaning (KRD) and at one year of age (KRA). Relative growth rate (RGR) was calculated using the method of Smith and Cundiff (1976). Computationally, the relative growth rate is the difference in natural logarithms of the initial and final weights divided by the time elapsed between the weights. Relative growth rate was calculated from birth to 205 days of age (RGRD), from 205 d of age to 365 d of age (RGRA). The variables were analyzed with a general linear model with PROC GLM (SAS, 2013) that included the fixed effects of herd, year and time of birth, sex of animal and dam's age at calving as a covariate. Correlations between traits were estimated with PROC CORR

Results

Males were 3.6, 5.6 and 8.8 heavier ($p<0.05$) for birth weight, adjusted weaning at 205 and 365 days, respectively. Females were 1.1, 8.7, 1.9 and 9.1% ($p<0.05$) lower for RGRD, RGRA, KRD and KRA, respectively. Animals weaned in October - December and January - March had lower KRD than those born in other seasons. No differences ($P>0.05$) were found in KRA at any of the times studied. Females had 1.1, 8.7, 1.9 and 9.1% lower ($P<0.05$) performance for RGRD, RGRA, KRD and KRA, respectively. Birth weight correlations with PA205 and PA365 were low and positive, with KRD and RGRD negative and with KRA and RGRA practically zero. The highest correlations were found between PA205 and KRD (0.80), PA205 and RGRD (0.72), KRD and RGRD (0.94) and KRA and RGRA (0.99). Environment is an important non-genetic factor, which influences the phenotypic expression of the different growth measures, their Kleiber indices and relative growth rate at different ages.

Conclusions

Environmental factors are significant sources of variation in growth and efficiency traits at one year of age and play an important role in expression of genetic potential. The results of this work show that adjusted weight at 205 and 365 days are important traits to consider for improving cattle productivity. The existence of high correlations between adjusted weight at 205 and 365 days with their corresponding Kleiber indices and relative growth rate suggests that selection for increased growth rate over either of these two age intervals, either in absolute or relative terms, would tend to alter the shape of the growth curve over the selection interval.

HERD HEALTH AND PRODUCTION MEDICINE

1001 - Associations between milk and blood biochemical parameters and metabolic status in dairy cows during lactation

Author: Radojica Djokovic, Marko Cincovic, Biljana Andjelic, Milos Petrovic, Aleksandar Cukic

Objectives

This study aimed to determine blood and milk metabolic parameters and their relationships for the purpose of evaluating the metabolic status of dairy cows for early diagnosis of subclinical metabolic diseases at different stages of lactation.

Material and methods

Blood and milk samples were collected from 100 Holstein dairy cows during morning milking. The cows were allocated to four groups according to the production period, including cows in early (from 1 to 49 days, *n* = 18), full (50 to 109 days, *n* = 26), mid (110 to 209 days, *n* = 25) and late (210 to 305 days and more, *n* = 31) lactation. The value of non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), glucose, triglycerides (TG), total cholesterol (TChol), total protein (TP), albumin, globulin, urea, total bilirubin (TBil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and lactate dexydrogenase (LDH) in the blood were determined by colorimetric kits (Biosystem, Spain and Randox, Carlisle, UK) and a Chemray spectrophotometer (Rayto, Shenzhen, China). The milk samples were analyzed by a FOSS milk analyzer, and their chemical composition (fat, protein and lactose) was assessed by a MILKOSCANFT analyzer (Milko-Scan 133 B, Foss Electric, Denmark). Upon serum separation, milk was subjected to biochemical tests for the determination of the enzymes (AST, ALT, ALP, GGT, LDH), urea and BHB. The biochemical reagents and apparatus used for milk serum analysis were the same as for blood serum. The effect of lactation period on blood and milk biochemical parameters was examined by ANOVA analysis coupled with an LSD post hoc test. Associations between milk and blood biochemical parameters were determined by Pearson's coefficient of correlation and regression analysis. SPSS statistics software (IBM, USA) was used. The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

Results

Blood serum NEFA, BHB, TBil, AST, ALT, ALP and LDH were higher ($p < 0.01$) in early lactation cows, whereas glucose, TG, TP, globulin and urea levels were significantly lower ($p < 0.01$) in early lactation cows than in other groups of cows. This results suggest that early lactation cows showed physiological adaptive changes, which were associated with subclinical ketosis and mild fat infiltration of liver cells. Milk fat and lactose levels were lower ($p < 0.05$) in early lactation cows, whereas milk protein and the activities of AST, ALT, ALP and LDH in milk serum were greater ($p < 0.05$) in early lactation cows. Milk fat was positively correlated with glucose, TP and TG, and negatively correlated with BHB, NEFA, TBil, ALT, LDH and ALP levels in the blood ($p < 0.05$). Enzyme activities in milk were positively correlated with those in blood and with blood NEFA, BHB and TBil levels, and negatively correlated with blood glucose, TChol and TG ($p <$

0.05). A significant positive correlation existed between blood and milk BHB values ($p < 0.05$). These results were also confirmed by the regression lines for the relationships milk fat:blood BHB, milk fat:blood NEFA, milk BHB:blood BHB, which showed good homogeneity with similar slopes through the four stages of lactation. These correlation and regression relationships clearly indicate that milk fat was directly correlated with blood glucose, protein and TG over the entire course of lactation, and negatively correlated with the lipomobilization and NEB (negative energy balance) parameters (NEFA, BHB), i.e.,indicators of the functional and morphological state of the liver (enzymes, bilirubin). The results are showing that milk fat content in dairy cows during the first months of lactation can be monitored with moderately high accuracy using routine milk measurements

Conclusions

The similar changes in blood and milk metabolites concentrations during lactation and milk to blood correlations confirm that milk has great potential in predicting of blood metabolites and metabolic status of cows.

1002 - Examination the metabolic status of dairy cows during early lactation with different revised quantitative insulin sensitivity check index

Author: Radojica Djokovic, Marko Cincovic, Milos Petrovic, Biljana Andjelic, Aleksandar Cukic

Objectives
The revised quantitative insulin sensitivity check index (RQUICKI) is the most commonly used indicator of insulin resistance in dairy cows. The aim of this study was to examine the characteristics of metabolic status in cows with different RQUICKI index values during early lactation.
Material and methods
The experiment was performed on 40 Holstein-Friesian cows without a health disorders during the previous lactations. Blood samples were taken in the first week after calving by puncture of the v. caudalis mediana, between 11:00 and 14:00 hours. The ELISA technique (Rayto, Shenzhen, PRC) was used to determine the following hormones: insulin, insulin like growth factor I (IGF-I), cortisol, somatotropic hormone (STH), triiodothyronine (T3) and thyroxin (T4). The blood concentrations of glucose, beta-hydroxybutirate (BHB), non-esterified fatty acids (NEFA), total proteins (TP), urea, albumin, cholesterol, triglycerides (TG), total bilirubin (tBil.), aspartate-aminotransferase (AST), alanin-aminotransferase (ALT), calcium (Ca), inorganic phosphorus (iP) and malondialdehyde (MDA) were determined using spectrophotometric method (Rayto, Shenzhen, PRC). Evaluation of body condition score (BCS) was performed at the moment of blood sampling according to Elanco Animal Health Bulletin Al 8478 (rev 9/96). The RQUICKI index was calculated according to the formula: RQUICKI = 1 **/** [log (glucose mg/dl) + log (insulin µU/ml) + log (NEFA mmol/l)]. Upper, median and lower quartiles for the RQUICKI index were determined. Cows were divided into four quartiles (Q1-Q4); Q1 RQUICKI index below the lower quartile - most resistant to insulin; Q2 RQUICKI index between the lower and median quartiles; Q3 group RQUICKI index in a range from the median to the upper quartile; Q4 group of cows had values in the range of the upper quartile to the maximum values - most sensitive to insulin. The cows were divided into four groups with 0, 1, 2 and ≥ 3 metabolic abnormalities, according to the cut-off values of the blood parameters: glucose < 2.5 mmol/l, NEFA >0.6 mmol/l, BHB >1 mmol/l, AST > 100 IU/l, TG < 0.11 mmol/l, tBil. > 8 µmol/l, Ca < 2.1 mmol/l, T3 < 1 nmol/l, T4 < 30 nmol/l. The influence of the classification of cows on the concentrations of the selected metabolic parameters was examined by ANOVA method and LSD test. The values of the metabolic parameters have regressed as a function of the value of the RQUICKI index in order to obtain intercepts and slopes: one for cows in Q1, and one for cows in the Q2-4 groups. Differences in slopes and intercept between the two groups were calculated using a *t*-test. The statistical software Statgraphics Centurion (Statpoint Technologies Inc. Warrenton, USA) and Excel (Microsoft, USA) were used for statistical analysis. The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).
Results
Metabolic parameters were significantly different in early lactation cows, classified according to the values of the RQUICKI index. The cows that were the most resistant to

insulin (Q1) had higher levels ($p<0.05$) of NEFA, cortisol, STH, BHB, tBil., AST, MDA and BCS in comparison to the cows that were the least resistant to insulin (Q4). The cows also had lower levels ($p<0.05$) of IGF-I, T3, T4, albumin, cholesterol, TG, Ca and iP as well as a tendency towards lower insulin and glucose concentrations. Metabolic parameters were strongly regressed ($p<0.01$) by RQUICKI in the most insulin resistant cows (Q1) in relation to the cows in the other groups, Q2-4. The cows with a higher number of metabolic abnormalities in their metabolic profiles had lower ($p<0.05$) RQUICKI values: 0.56 ± 0.045 (no abnormalities); 0.52 ± 0.041 (1 abnormality); 0.47 ± 0.042 (2 abnormalities) and 0.40 ± 0.043 (≥3 abnormalities).

Conclusions

The cows with a lower RQUICKI index and high BCS showed metabolic adaptations that indicated a catabolic load on the organism, the development of ketosis, fatty liver, ion imbalances and oxidative stress. Metabolic parameters were strongly regressed by RQUICKI in the most insulin resistant cows in the first week of lactation. The cows exhibiting a higher number of metabolic abnormalities had lower RQUICKI index values. The RQUICKI index could be a potent indicator of metabolic status in cows during early lactation.

1018 - IgG content and bacteriological quality of colostrum from Dutch Holstein-Friesian and Belgian Blue cows

Author: Geert Hoflack, Pleun Penterman, Geert Vertenten, Wim Schaap, Frank van Hagen, Bart Sustronck, Eva Haas

Objectives

Adequate transfer of passive immunity is essential in the bovine species and can only be achieved when good quality colostrum is used. Colostral immunoglobulin G (IgG) concentration and bacterial contamination of colostrum have been shown to vary between cows, but comparative data on different breeds are scarce. The objective of the present field study was to investigate the IgG concentration and the total bacterial cell count in colostrum from Dutch Holstein Friesian (HF) and Belgian Blue cows.

Material and methods

Colostrum samples (n= 162) were collected from 88 commercial farms located in Belgium and The Netherlands. All the samples from the Belgian farms were from Belgian Blue cows (n= 76) whereas the samples from The Netherlands were all from HF cows (n=86). All samples were collected within the first 6 hours after parturition and frozen at -20°C until processing. To quantify the IgG concentration in colostrum, a commercial competitive ELISA-test kit (BIO K420, MonoScreen QuantELISA Immunoglobulin Easy, Bio-X Diagnostics S.A., Rochefort, Belgium) was used. Indirect evaluation of the IgG concentration of the colostrum samples was performed using a digital Brix refractometer (Milwaukee Refractometer MA871, Milwaukee Instruments Inc., NC, USA). Total bacterial cell count of the colostrum samples was determined using a bactoscan automatic bacterial count reader (Bactoscan™ FC+, FOSS, Denmark). All statistical analyses were performed in R (R Core Team, 2017). Comparison of samples from Dutch HF cows and Belgian Blue cows was performed. Results were considered as significantly different for $P \leq 0,05$.

Results

The colostral IgG concentration of Belgian Blue cows (mean: 81,24 ± 24,90 g IgG/l; range: 22,41 - 150,00 g IgG/l) was significantly (t_{Welch}; $p \leq 0,001$) higher than that of the HF cows (mean: 53,19 ± 17,61 g IgG/l; range: 14,4 - 99,07 g IgG/l). Using an IgG concentration of at least 50 g/l as cut-off to determine good quality colostrum, only 8,22 % of the colostrum samples from Belgian Blue cows were of poor quality compared to 34,5 % of the colostrum samples from Dutch HF cows. The mean Brix refractometric value for colostrum from Belgian Blue cows was 25,27 ± 4,05 % (range: 16,77 – 36,40 %) whereas this was 21,10 ± 4,21 % (range: 9,33 – 29,27 %) for Dutch HF cows. This difference was statistically significant (t_{Welch}; $p \leq 0,001$). When considering the ELISA technique as golden standard, an optimal cut-off value of 21,9 % (Se 0,78 [$CI_{95\%}$ 0,61 - 0,90]; Sp 0,762 [$CI_{95\%}$ 0,67 - 0,83]; AUC 0,79 [$CI_{95\%}$ 0,69 - 0,88]) to distinguish good from poor quality colostrum using the Brix refractometer method was obtained. When using this Brix refractometer cut-off value, 17,8 % of the Belgian Blue colostrum samples and 52,4 % of the Dutch HF colostrum samples, respectively, would be of poor quality. The bactoscan values of the colostrum samples were highly skewed and were therefore analysed using non-parametric methods. The median total bacterial cell count of the colostrum samples from the Belgian Blue cows was 5.500 CFU/ml (range: 4.000 – 390.000 CFU/ml). The colostrum samples from the Dutch HF cows had a median total bacterial cell count of

9.000 CFU/ml (range: 4.000 – 1.000.000 CFU/ml). This difference was statistically significant (Mann-Whitney U; $p \leq 0{,}001$).

Total bacterial cell count was above the norm of 100.000 CFU/ml in 5,48 % of the colostrum samples from Belgian Blue cattle, whereas 14,3 % of the colostrum samples from Dutch dairy cows were above this critical norm. Applying both the IgG content (at least 50 g IgG/l) and the total bacterial count (less than 100.000 CFU/ml) as criteria for good quality colostrum, 13,7 % of the colostrum samples from the Belgian Blue cows and 38,1 % of the colostrum samples from the Dutch HF cows were inadequate to be fed to new-born calves.

Conclusions

Belgian Blue cows have significantly higher colostrum quality in comparison to Dutch HF cows. Not only do Belgian Blue cows have a higher colostral IgG content, the bacterial load of their colostrum is also significantly lower compared to the colostrum from Dutch dairy cows.

1030 - Farm-level risk factors associated with increased milk β-hydroxybutyrate and ketosis prevalence on farms with automated milking systems

Author: Trevor DeVries n/a, Brandon Van Soest, Todd Duffield, Michael Steele

Objectives
The objectives of this study were to determine the farm-level ketosis prevalence, as diagnosed from milk β-hydroxybutyrate (BHB), on Canadian dairy farms milking with an automatic milking system (AMS) and to describe the farm-level housing, management, and nutrition risk factors associated with increased milk BHB and the within-herd ketosis prevalence in the first 45 days in milk (DIM).
Material and methods
Canadian AMS farms (n = 162; eastern Canada n = 8, Quebec n = 23, Ontario n = 75, western Canada n = 55) were visited once between April to September 2019 to record housing and herd management practices. The first test milk data for each cow under 45 DIM were collected, along with the final test of the previous lactations for all multiparous cows, from April 1, 2019 to September 30, 2020. The first test milk BHB was then used for each individual cow to classify as having subclinical ketosis (≥0.15 mmol/L) at time of testing. Milk fat and protein content, milk BHB, and ketosis prevalence were summarized by farm and lactation group (all, primiparous, and multiparous). During this same time period, formulated rations for dry and lactating cows, including ingredients and nutrient content, and AMS milking data were collected. Data from the AMS were used to determine milking behaviors and milk production of each herd during the first 45 DIM. Multivariable regression models were used to associate herd-level housing, feeding management practices, and formulated nutrient composition with first test milk BHB levels and within-herd ketosis levels separately for primiparous and multiparous cows.
Results
The within-herd ketosis prevalence for all cows was 21.8%, with primiparous cows having a lower average prevalence (12.2 ± 9.2%) than multiparous cows (26.6 ± 11.3%). Milk BHB (0.095 ± 0.018 mmol/L) and ketosis prevalence for primiparous cows were positively associated with prepartum dry matter intake (DMI) (P=0.001) and forage content (P=0.002) while being negatively associated with postpartum DMI (P=0.001), the major ingredient in the concentrate supplemented through the AMS (P=0.02), and the PMR-to-AMS concentrate ratio (P=0.001). However, multiparous cows' milk BHB (0.123 ± 0.023 mmol/L) and ketosis prevalence were positively associated with the length of the previous lactation (P=0.03), milk BHB at dry off (P<0.001), prepartum diet nonfiber carbohydrate content (P=0.06), and the major forage fed on farm (P=0.02), while tending to negatively associate with feed bunk space (P=0.09) in the lactation period.
Conclusions
This is the first study, to our knowledge, to determine the farm-level risk factors associated with herd-level prevalence of ketosis in AMS dairy herds, thus helping optimize management and guide ration formulation to promote the reduction of ketosis prevalence.

1031 - Postpartum excretion of internal teat sealant after selective dry cow treatment of dairy cows

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Objectives
To comply with antibiotic restriction policies in the European Union, Internal Teat Sealants (ITS) are increasingly used at drying off (DO) in selective dry cow treatment protocols to maintain udder health. Our objective was to improve insight in ITS excretion visibility and to compare quantity, pattern and presence *versus* absence of ITS excretion post-calving between the typical two cow categories at DO: High and Low SCC cows.
Material and methods
In herds in the Netherlands (n=3), and Germany (n=4), cows were enrolled at DO, and categorized as High SCC (n=93) or Low SCC (n=99). All quarters of all cows received ITS at DO, only High SCC cows were additionally treated with antibiotic in all quarters. Post-calving, ITS visibility, quantities, patterns and percentage of ITS infused at DO and excreted post-calving were recorded from 50 mL of pre-milk of every quarter at each of the first 15 or 16 milkings. Udder health status was determined by bacteriological culture and somatic cell counting of quarter milk samples taken at DO and at day 3 post-calving and by clinical mastitis incidence from DO until 30 DIM. An univariable and multivariable model were created to explore associations of ITS excretion presence *versus* absence at the first 3 milkings.
Results
Irrespective of SCC category at DO, both laboratory personnel, and farmers saw ITS residues at the first milking in an equal 72% of quarters. At subsequent milkings, laboratory personnel saw ITS residues in 6-20% more quarter samples compared to farmers. At the first milking, ITS excretion quantities showed a bimodal distribution pattern and the mean percentage of ITS infused at DO (3.83 g) that was excreted in pre-milk at the first milking, was higher in the Low (45.5%) compared to the High SCC cow category (32%) at DO. At the second and third milking, mean adjusted ITS percentage excreted was higher in the High (8.5% and 1.8%, respectively) compared to the Low SCC category at DO (4.6% and 0.4% respectively). The multivariable model showed only parity was significantly associated to ITS presence at the first and second milking. When corrected for parity, ITS residues were more often present in Low SCC cows at DO at the first milking, and more often present in High SCC cows at DO at the second and third milking. An additional univariable model showed no association between ITS presence at the first milking and udder health.
Conclusions
In conclusion, in pre-milk of the first milking, ITS residue excretion was bimodal, higher in Low SCC cows, more likely present in multiparous cows, and not associated with udder health. At the second and third milking, excretion was higher in High SCC cows and ITS presence was only more likely in multiparous cows at the second milking.

1037 - Herd Planning Service for veterinarians using on-farm automated monitoring systems an effectual marketing pilot study

Author: Liz Cresswell, Juan Pedro Campillo Beneitez, Monica Miravalle, Monika Ptaszynska-Sutton

Objectives

Automated behavior monitoring systems (ABMS) are an example of on-farm monitoring technologies which measure different behaviours, such as rumination and activity to provide insights to farmers that can be used to make decisions regarding health and reproduction. However, such insights are often not used or underestimated. Previous research has demonstrated that veterinarians are trusted farm advisors. Data produced by monitoring technologies are an opportunity for veterinarians to add value to these insights, yet feedback from the field indicated that few veterinarians are engaging with on-farm monitoring technologies on a regular basis.

The objective of this pilot project was to employ an effectual marketing strategy to initiate and gain feedback on a new service which trains veterinarians in optimising the use of data insights produced by an ABMS (SenseHub™ Dairy, MSD Animal Health) in order for them to use the data as part of their routine herd planning.

Material and methods

Effectual marketing is a strategy used to actively learn from a market and inform future operations. It was employed to pilot this new service with eight dairy veterinarians, each with one vet-nominated dairy farmer across the south of England between August and October 2023. They were selected using convenience sampling based on previous expressed interest and prior installation of an ABMS (SenseHub™ Dairy).

The ABMS (Sensehub™ Dairy) is a cattle monitoring system which uses real-time rumination, activity, eating and milk data to produce insights about individual and herd-level that can be used to make decisions regarding, reproduction, health, nutrition and milk production.

After consent was obtained, the herd planning service was piloted in three parts:

10. A Data Collection Form (DCF) was completed by the farmer, collecting information on:

- Basic farm information
- Farm goals and challenges
- Disease and vaccination status.

11. The farmer's vet undertook a bespoke training session on the use of the ABMS (Sensehub™ Dairy) with a Veterinary Technical Advisor [MSD Animal Health]. Using the farm goals identified on the DCF, data insights produced by the ABMS were used to create three to five herd action points.

12. A meeting between the selected veterinarian, trainer and farmer was held to review the training and agree a timeframe for achieving the specified actions.

Real-time feedback was recorded in writing during each of the meetings and thematic analysis used to analyse the results of the pilot study. Feedback was coded and categorised by theme ('category codes').

Results

The three main feedback themes obtained were identified as follows, with category codes listed in order of frequency of mention by participants –

- Improving herd health – specific data insights applicable to improving herd health on the participating farms were identified. Category codes: fertility gains, improving submission rate, transition management, group management and calving pattern.
- Needs of the service – category codes: early involvement of the veterinarian, providing standard operating procedures, understanding programme interoperability, providing an evidence base, resolving technical issues.
- Role of the veterinarian – category codes: changed medicine purchasing habits, reducing emergency calls, providing remote support and involving other consultants.

Conclusions

An effectual marketing approach was used to obtain feedback from veterinarians and farmers on a pilot study of the herd planning service. Feedback was coded and categorised and three main themes were identified – improving herd health, the needs of the service and the role of the veterinarian.

Specific feedback will be used to inform the development of the herd planning service and develop supporting materials e.g. standard operating procedures and a tailored evidence base. To the authors' knowledge this is first pilot study of a service which trains veterinarians in optimising the use of data insights produced by an on-farm monitoring technology in herd planning.

1041 - Antimicrobial resistance of Escherichia coli from dairy farms participating in an antimicrobial stewardship educational program for farm employees

Author: Adriana Garzon Audor, Rafael Portillo, Gregory Habing, Noelia Silva-del-Rio1, Betsy Karle, Richard Pereira

Objectives

Antimicrobial use in food-producing animals is under increasing scrutiny due to the potential impact on the selection of antimicrobial-resistant bacteria that may be transmitted to humans by direct contact, with the food chain, or the environment. Novel data monitoring commensal *E. coli* from dairy farms is essential for understanding antimicrobial resistance (AMR) patterns and their association with herd health management practices. The objectives of this study were to: 1) compare the prevalence of antimicrobial resistance in the *E. coli* isolates from the hospital, fresh, and mid-lactation pens from 18 conventional dairy farms participating in an educational training program in antimicrobial stewardship practices in California and Ohio, and 2) to characterize the prevalence of antimicrobial resistance of commensal *E. coli* isolated from pooled fecal pat samples before and 3 months after participating in the educational training program.

Material and methods

Pooled fecal pat samples were collected from the hospital pen, the fresh pen (1 to 5 DIM), and the mid-lactation pens (90 to 150 DIM) on conventional dairies in CA (n=9) and OH (n=9). Fecal samples were collected as part of a larger study using a quasi-experimental design that assigned farms to the training intervention group (TG; 9 per state) or the control group (CG; 3 per state). For the TG, farm worker(s) identified as having the task of diagnosis and treatment of adult cows on the farm participated in a training program on antimicrobial stewardship practices. Pooled fecal samples (n=7) were collected at enrollment and three months after completing the intervention on each of the participating farms (n=18), followed by culture for *E. coli* isolation and antimicrobial sensitivity testing using the broth microdilution methodology. Logistic regression models were used to evaluate the association between *E. coli* antimicrobial resistance patterns with the training intervention and farm-level factors.

Results

No effect was observed in the prevalence of resistant isolates between the control and intervention farms after the training was delivered. Isolates from the hospital pens were 2.48 (95% CI: 1.06 – 6.22, $P = 0.03$) and 5.61 (95% CI: 1.94 – 16.91, $P < 0.001$) times, more likely to be resistant to streptomycin and chloramphenicol, respectively, than isolates from the mid-lactation pens.

Conclusions

Our findings indicate there was a higher prevalence of AMR in *E. coli* associated with the hospital pen within the farm, while the training program for three months did not affect the prevalence of AMR in *E. coli* on the farms participating in the program. Further research efforts should be conducted to identify factors driving AMR at the pen level, as well as approaches that could be used to reduce the risk of disseminating AMR from sick pens to animals being housed and to other pens on the farm.

1044 - Sensitivity and specificity of an in-line somatic cell count sensor in assessing milk quality compared with milk culture at the end of lactation in dairy cows

Author: Scott McDougall, Rob Orchard, Amanda Kilby, Jo Holter, Juan Pedro Campillo, Jantijn Swinkels, Tiago Tomazi

Objectives

Minimizing the use of antimicrobials at the end of lactation (dry cow therapy) requires categorization of cows as likely infected or uninfected. While microbiology is the gold standard for such categorization, the costs of doing so mean that indirect tests such as somatic cell count (SCC) are commonly used. An in-line SCC sensor (SenseHub™ In Line Somatic Cell Count Sensor; **in-line SCC; MSD Animal Health**) is commercially available but its *utility in differentiating cows eligible for dry cow therapy at the end of lactation has not been assessed*.

Thus, the objective of this prospective diagnostic accuracy study was to define the sensitivity (**Se**) and specificity (**Sp**) of the in-line SCC against a cow-composite milk samples submitted for conventional microbiology. A secondary objective was to assess the utility of the in-line SCC compared with the maximum herd test (HT) (**max HT SCC**) or last (**last HT SCC**) SCC determined from cow-composite milk samples collected as part of routine herd production recording at monthly intervals throughout lactation.

Material and methods

Cows (n=1,544) from four spring-calving, predominantly pasture-fed, dairy herds from three regions of New Zealand had cow-composite milk samples collected following aseptic teat-end preparation immediately prior to or after the final milking of lactation. These samples were submitted for routine microbiology. The microbiology data from approximately half the cows (n=770; training data set) were randomly selected after blocking for intramammary infection (**IMI**) status within herd and these data were used to determine the optimal predictor derived from the in-line SCC data selected from the maximum, geometric mean, and bounded geometric mean in-line SCC over the last 1, 2, 4, 6, 8, and 12 weeks of lactation, by selecting the predictor with the maximum area under the receiver operator curve (**AUC ROC**). The bounded geometric mean SCC over the final twelve weeks (**in-line 12wSCC**) of lactation was found to be the best predictor for the further analyses. The term “bounded” in this instance means that in-line SCC results less than 50,000 cells/mL were replaced with a value of 50,000 cells/mL prior to averaging.

The **Se** and **Sp** of the in-line 12wSCC for any IMI or for a major pathogen IMI (defined as presence of *Staphylococcus aureus*, *Streptococcus dysgalactiae* or *Streptococcus uberis*) was calculated using the test data set (n=774). The AUC for the max and last HT SCC was formally tested against that of the in-line 12wSCC.

Results

The cow-level prevalence of any IMI or a major IMI across all cows were 50.6% and 14.2%, respectively. The Se and Sp of the in-line 12wSCC for any IMI was 0.68 (95% CI 0.64-0.72) and 0.71 (95% CI 0.65-76), respectively, and the Se and Sp for a major pathogen IMI was 0.89 (95% CI 0.82-0.95) and 0.51 (95% CI 0.47-0.55), respectively, at a cut-point of 150,000 cells/mL.

The AUC for a major pathogen IMI was 0.82 (95% CI 0.79-0.86), 0.82 (0.78-0.86) and 0.84 (95% CI 0.90-0.97) for in-line 12wSCC, max HT SCC and last HT SCC, respectively. These AUC did not differ and the AUC for the in-line 12wSCC was non-inferior to that of the max and last HT SCC (both $P < 0.001$).

Conclusions

It was concluded that the 12-week bounded geometric mean in-line SCC had an AUC, Se and Sp not different from that of the last or maximum herd test SCC and hence this test has utility in differentiating those eligible cows for implementing dry cow therapy treatments.

1046 - Colostrum collection using robotic milking systems: impact on microbiological quality

Author: Pleun Penterman, Geert Hoflack, Geert Vertenten, Wim Schaap, Bart Sustronck, Linde Gille

Objectives

Calves are agammaglobulinaemic at birth and rely on the uptake of immunoglobulins from their dam's colostrum shortly after birth. This absorption of immunoglobulin G from the colostrum by the calf can, unfortunately, be hampered by a high bacterial load of that colostrum. Hence, strict hygienic procedures when collecting, storing and feeding colostrum are of paramount importance for a good transfer of passive immunity in bovine neonates. The aim of the present study was to evaluate whether the use of robotic milking systems for the collection of bovine colostrum could influence the degree of bacterial contamination of bovine colostrum.

Material and methods

A total of 76 paired colostrum samples from 38 HF cows were collected on 3 different farms in the Netherlands. From each cow a hand-milked four quarter colostrum sample was collected, discarding the first 4 to 6 squirts of colostrum from each teat, within 2 hours after calving and immediately before the cow was allowed to the robotic milking system. The colostrum of each cow was thereafter harvested in a clean bucket using the separation system of the milking robot. An aliquot of 100 ml was collected from the separation bucket for further analysis. All samples were stored at -20°C until processing. The total bacterial cell count (TBCC) and total coliform count (TCC) were determined at 22°C using ISO 6222 standard methods. TBCC and TCC values were Log10 transformed before analysis. Mixed linear regression models, with cow as random effect, were constructed to evaluate the influence of the robotic milking system on the TBCC and TCC of the colostrum. Additionally, the influence of farm on both TBCC and TCC of colostrum was evaluated. Statistical analysis was performed in R (R Core Team 2022).

Results

Hand milked colostrum samples had a lower mean TBCC (11e4 cfu/ml) and TCC (6e1 cfu/ml) than those collected from the separation bucket after robotic milking of the cows (TBCC of 1e6 cfu/ml and a mean TCC of 11e4 cfu/ml).

Hand milked colostrum samples were associated with a significantly lower TBCC (-5.59 Log10 cfu/ml; 95% CI -6.26 to -4.92 Log10 cfu/ml; $p<0.001$) than those collected by robotic milking. Additionally, colostrum samples collected directly from the udder were associated with a significantly lower TCC (-2.52 Log10 cfu/ml; 95% CI -2.91 to -2.13 Log10 cfu/ml; $p<0.001$) compared to colostrum samples collected from the separation bucket after robotic milking of the cows.

The degree of bacterial contamination, both TBCC and TCC, was significantly associated with the farm on which the samples were collected ($p<0.001$).

Conclusions

The results of this study clearly indicate that the microbiological quality of colostrum is significantly reduced when robotic milking systems are used for colostrum collection. An exact reason for this remains unclear, but insufficient or difficult cleaning after passage

of the viscous colostrum and of mastitic milk in the separation lines of the robotic milking system could be at the base of this observation. Moreover, the study demonstrated that the degree of this negative effect of robotic milking on the microbiological quality of the colostrum is farm dependent.

When using robotic milking to harvest colostrum the necessary hygienic measures (e.g. cleaning and disinfection) should be applied to prevent excessive bacterial contamination during colostrum collection. Ideally, colostrum is hand milked to avoid this contamination.

1048 - Effect of storage conditions and initial bacterial load on microbiological quality of colostrum

Author: Pleun Penterman, Geert Hoflack, N. Botteldoorn, Wim Schaap, Geert Vertenten, Bart Sustronck, Eva Haas

Objectives

Colostrum is the first milk secreted after calving and contains high quantities of immunoglobulin G (IgG), which is necessary for the newborn calf to obtain passive protection against disease. Absorption of colostral IgG's can be hampered by the bacterial content in the colostrum. Colostrum storage conditions may alter the bacterial concentration in colostrum over time and may negatively impact the absorption of IgG's by the newborn calf. Therefore, the objective of this study was to measure the effect over time of storing colostrum in different conditions on the Total Bacterial Cell Count (TBCC) and the Total Coliform Count (TCC) in colostrum of Holstein-Friesian cows in the Netherlands. Additionally, the influence of the initial bacterial load of the colostrum samples at the start of the storage period on the evolution of the TBCC and TCC was evaluated.

Material and methods

This study was part of a larger study in which 76 paired colostrum samples were collected from 38 Holstein-Friesian cows originating from 3 different farms in the Netherlands. A random selection of colostrum samples from this larger study was processed in the current study. On the one hand, five samples with a low TBCC (≤1,000 cfu/ml) and a low TCC (≤10 cfu/ml) (low initial bacterial load) and on the other hand, five samples with a high TBCC (≥1,000,000 cfu/ml) and high TCC (≥1,000 cfu/ml) (high initial bacterial load) were randomly selected for the current colostrum storage study. Aliquots of all the selected colostrum samples were stored for up to 120 hours in temperature-controlled units set at 4, 22, and 30 °C. All colostrum samples were analysed for TBCC and TCC after 0, 24, 48, 72, 96 and 120 hours of storage. TBCC and TCC were determined at 30°C using standard accredited bacteriological protocols. Log transformation of the data was applied in case of non-normal distribution. Repeated measures ANOVA with post-hoc Tukey HSD testing was performed to assess the evolution of the TBCC and TCC over time at different storage temperatures. Statistical analysis was performed in R (R Core Team 2022).

Results

In the colostrum samples stored at 4°C the TBCC and TCC showed a minor non-significant increase after 96 to 120 hours of storage. When taking only the samples with a low initial bacterial load into account, the TBCC and TCC remained constantly low during the complete storage period. However, when considering the samples with a high initial bacterial load, a significant increase of the TBCC was seen after 72, 96 and 120 hours of storage at 4°C. The TCC in the samples with a high initial load remained constant when stored at 4°C for 5 days.

Storage of colostrum at 20°C resulted in a significant increase of both the TBCC and TCC after 24 hours of storage. The TBCC continued to increase reaching its peak after 48 hours of storage. Thereafter, the TBCC plateaued at a high level during the remaining storage period. The same pattern in TBCC was seen in both high initial bacterial load samples and low initial bacterial load samples. TCC growth pattern in low initial bacterial

load samples was similar to the TBCC pattern. However, the TCC in high initial bacterial load samples increased more rapidly reaching its peak at 24 hours of storage, whereafter a decline in TCC was observed during the remaining storage period.
At a storage temperature of 30°C the TBCC and TCC of colostrum increased sharply reaching their peak after 24 hours of incubation, both the TBCC and TCC then plateaued for the next 24 hours of storage after which a decline in TBCC and TCC was observed. In the samples with a high initial bacterial load this decline after 48 hours of storage was more pronounced.

Conclusions

Storage of colostrum at 4°C for 5 consecutive days only has a minor effect on the TBCC and TCC of that colostrum. However, if colostrum with a high initial bacterial load (TBCC ≥1,000,000 cfu/ml and TCC ≥1,000 cfu/ml) is stored at 4°C for more than 48 hours, a significant increase in TBCC and TCC may occur, indicating that besides the storage of colostrum at an appropriate temperature, hygienic harvesting of colostrum is of utmost importance for its microbiological quality. Storing colostrum at higher temperatures then at 4°C should be avoided.

1053 - Clinical, hematological and lung evaluations using metaphylactic strategies with tildipirosin for the control of bovine respiratory disease in feedlot cattle

Author: Pedro Rodriguez Fernandez, Andre Pacheco de Carvalho, Henderson Ayres, Amauri Alcindo Alfieri, Selwyn Arlington Headley, Aline Barichello Cerqueira, Roberta Dias da Silva Cunha, Anderson Lopes Baptista, Paulo Henrique Jorge da Cunha

Objectives

Metaphylaxis is the practice of administering an approved antimicrobial to an entire lot or pen of cattle with the intent of controlling the incidence of Bovine Respiratory Disease (BRD). The study evaluated the effects of a metaphylactic protocol for BRD on the clinical, hematological, and pulmonary outcomes of high-risk feedlot cattle.

Material and methods

Two hundred eight high-risk Crossbred bulls (*Bos taurus × Bos indicus*), aged between 24 and 30 months, from a commercial feedlot farm, located in Frutal, Minas Gerais, Brazil, were included in the study. High-risk cattle met the enrolment criteria, which included animals being acquired from an external source, having a body condition score between 1 and 2 on a scale of 1 to 5 and having traveled over 500 km for more than 8 hours with three days of resting upon arrival at the feedlot. Cattle were divided randomly into two groups, and at the time of processing, the *Met-Group* (n=104) received a single subcutaneous administration of tildipirosin (Zuprevo®, MSD Animal Health) at a dose of 4 mg/kg BW (0.022 mL/Kg BW). The *Control-Group* (n=104) received a subcutaneous administration of 0.9% saline at the same dose. Characterization of BRD was based on the DART system (nasal and ocular discharge, rectal temperature, ear and head carriage, coughing, and respiratory signs). A blood sample of all cattle with BRD clinical signs was obtained for hemogram. A macroscopic assessment of the lung tissue was initially carried out during the post-mortem inspection. The findings were photographed and recorded as a schematic representation, adapted from Sobestiansky (2001). To assess the distribution and severity of lung alterations, lesion scores were established based on the lung area affected as a percentage. Subclinical cases were considered as animals without clinical signs of BRD and macroscopic lung lesion at slaughter. These lesion scores were adapted from the method described by Griffin et al. (2010). The scores were classified as: grade 0 (no lesions), grade 1 (<10% of the lung with lesions suspicious of pneumonia), grade 2 (≥10% and ≤15% of the lung with lesions suspicious of pneumonia) and grade 3 (>15% of the lung with lesions suspicious of pneumonia and/or altered pulmonary lymph nodes). A descriptive analysis was carried out on the data obtained (pulmonary score system). Statistical analysis of the data was performed using the ANOVA test. Student's T test was applied to the hematological variables, with a 5% significance level using the R software.

Results

The BRD morbidity rate in *Met-Group* (8.65%) was lower than in *Control-Group* (23%). The main clinical signs observed in BRD animals were prostration, as well as nasal and eye discharge. No deaths were reported in either of the groups. There were no significant differences between groups ($p>0.05$) in the hematological parameters evaluated for cattle with BRD. In the *Met-Group*, 27 (25.94%) lungs with lesions were detected, but 9 (8.64%) were from animals with clinical signs and 18 (17.30%) were

subclinical cases. On the other hand, in *Control-Group*, 40 (38.46%) lungs with lesions were identified and 23 (22.11%) were from cattle with clinical signs and 17 (16.34%) were from subclinical cases.

Conclusions

This study concluded that the use of antimicrobial metaphylaxis with tildipirosin in high-risk feedlot cattle reduced the morbidity rate of BRD and resulted in a lower percentage of pulmonary alterations.

1058 - Ketosis during the post-calving transition period and hematological/serum biochemical parameter levels on calving day and ketosis onset in Holstein cows

Author: Seungmin Ha Justus, Seongsoo Hwang, Jihwan Lee, Seogjin Kang, Mooyoung Jung, Sang Bum Kim, Donghyeon Kim, Ki Choon Choi, Jinho Park

Objectives
This study aimed to evaluate the hematological and serum biochemical parameter levels on the calving day and the onset of ketosis according to ketosis severity in Holstein milking cows.
Material and methods
Blood samples were collected once every 3 days (eight times in 21 days) during the post-calving transition period. Cows were divided into three groups according to the highest β-hydroxybutyrate (BHBA) concentration in any of the eight samples.
Results
The non-ketosis (NK; BHBA<1.2 mmol/L; n=75) group had the highest red blood cell and monocyte counts and red cell distribution width and alanine transaminase levels and the lowest mean corpuscular volume, mean corpuscular hemoglobin, non-esterified fatty acid, and total bilirubin levels on the calving day and ketosis onset date, followed by the subclinical ketosis (SCK; ≥1.2 mmol/L and <3.0 mmol/L; n=46) and clinical ketosis (CK; BHBA ≥3.0 mmol/L; n=35) groups (p<0.05). The NK group had the highest changes in glucose, triglyceride, and magnesium, and the lowest changes in BHBA, lactate dehydrogenase, and aspartate transaminase levels from the calving date to the incidence of ketosis, followed by the SCK and CK groups (p<0.05).
Conclusions
Ketosis was associated with the values of RBC and monocyte counts; MCV, MCH, and RDW; and the levels of BHBA, NEFA, ALT, and TB both on the calving date and at the onset of ketosis during the post-calving transition period. Moreover, ketosis was associated with changes in the levels of BHBA, glucose, TG, AST, LDH, and magnesium from the calving date to the onset of ketosis. These findings may provide some insights into how ketosis develops during the post-calving transition period. However, further studies will be required to elucidate the mechanisms underlying the effects observed in this study.

1059 - Effects of subclinical mastitis on automatic milking system data, hematological and biochemical parameters, and milk composition in Holstein cows

Author: Seungmin Ha Justus, Mooyoung Jung, Seogjin Kang, Eunjeong Jeon, Dong-Hyun Lim, Donghyeon Kim Kim, Jin San Moon

Objectives

Subclinical mastitis causes damages in dairy industry due to decreased milk production, lowered milk quality, and high somatic cell counts (SCC) in milk despite normally appearing mammary gland and visibly normal milk. Cows with subclinical mastitis can be detected by automatic milking systems (AMS) at early stage. This study aimed to investigate changes in factors monitored by automatic health-monitoring and AMS before the incidence of subclinical mastitis and identify the differences in hematological and serum biochemical parameters and milk composition on the incidence of subclinical mastitis when detected by AMS.

Material and methods

A total of 32 Holstein cows were divided into two groups according to SCC of AMS and milk composition analysis and California mastitis test (CMT): healthy cows (CON, n = 16, <500 $\times 10^3$ cells/ml and negative to CMT) and cows with subclinical mastitis (SCM, n = 16, ≥500 $\times 10^3$ cells/ml and positive to CMT). In total, 60 milk samples from CON (mCON) and 61 milks samples from SCM were obtained and milk samples from SCM were divided into samples from non-inflamed quarters (mNQ, n = 34) and milk samples from inflamed quarters (mIQ, n = 27). We performed retrospective investigations on automatic health-monitoring and AMS, hematological, serum biochemical, and milk composition analyses, and bacterial isolation on milk.

Results

Rumination time, activity, and milk temperature were not associated with subclinical mastitis. CON maintained milk yield and constant electrical conductivity (EC) of milk. However, In SCM, milk yield had decreased and EC of milk had changed prior to the incidence; mNQ decreased EC of milk while mIQ increased EC (p<0.05). In hematological and serum biochemical parameters, SCM had higher globulin level and lower basophil counts, the levels of total cholesterol, albumin, the albumin-to-globulin ration and BUN than CON (p<0.05). In milk composition analysis, milk fat, milk protein, milk citrate, and milk solids were not associated with subclinical mastitis. mIQ had higher SCC but lower levels of milk lactose and milk solids not fat than mCON and mNQ (p<0.05). mCON had higher levels of milk non-protein nitrogen than mNQ (p<0.05). Opportunistic or environmental mastitis pathogens were isolated in mIQ.

Conclusions

This study demonstrates that changes in milk yield and EC of milk measured using AMS occurred prior to subclinical mastitis and subclinical mastitis may be associated with basophil counts, levels of total cholesterol, albumin, the albumin-to-globulin ration, blood urea nitrogen, globulin, SCC, milk lactose, and milk solids not fat on the incidence detected by AMS. These findings provide new insights into understanding subclinical mastitis at early stage.

1060 - Ketosis during the post-calving transition period and hematological/serum biochemical parameter levels on calving day and ketosis onset in Holstein cows

Author: Seungmin Ha Justus, Seogjin Kang, Mooyoung Jung, Sang Bum Kim, Seongsoo Hwang, Jihwan Lee, Donghyeon Kim, Ki Choon Choi, Jinho Park

Objectives
All dairy cows experience negative energy balance in the early lactation, however, the majority of cows do not develop ketosis. This study aimed to evaluate hematological and serum biochemical parameters on the calving date and the incidence of ketosis according to the severity of ketosis.
Material and methods
Blood sampling was conducted from the jugular vein of Holstein milking cows and β-hydroxybutyrate (BHBA) was tested from the calving date once every three days (8 times in 21 days) during the postcalving transition period. The cows were divided into three classifications according to the highest BHBA concentration observed from any of the eight samples as follows: non-ketosis (NK, <1.2 mmol/L, n = 75), subclinical ketosis (SCK, ≥1.2 mmol/L and <3.0 mmol/L, n = 46), clinical ketosis (CK, ≥3.0 mmol/L, n = 35). Hematological and serum biochemical analyses were performed.
Results
Regarding the calving date, the NK classification had the highest values of red blood cell (RBC), white blood cell, monocyte, and eosinophil counts, red cell distribution width (RDW), alanine transaminase (ALT), and lactate dehydrogenase (LDH), and the lowest values of BHBA, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), non-esterified fatty acids (NEFA), total bilirubin (TB), and magnesium, followed by the SCK and CK classifications ($p < 0.05$). Regarding the incidence of ketosis, the NK classification had the highest values of RBC and monocyte counts, RDW, triglyceride (TG), and ALT, and the lowest values of MCV, MCH, NEFA, and TB, followed by the SCK and CK classifications ($p < 0.05$). Regarding the changing extents between calving date to the incidence of ketosis, the NK classification the highest values of glucose, TG, and magnesium, and the lowest values of BHBA, LDH, and aspartate transaminase (AST), followed by the SCK and CK classifications ($p < 0.05$).
Conclusions
Ketosis was associated with the values of RBC and monocyte counts; MCV, MCH, and RDW; and the levels of BHBA, NEFA, ALT, and TB both on the calving date and at the onset of ketosis during the post-calving transition period. Moreover, ketosis was associated with changes in the levels of BHBA, glucose, TG, AST, LDH, and magnesium from the calving date to the onset of ketosis. These findings may provide some insights into how ketosis develops during the post-calving transition period. However, further studies will be required to elucidate the mechanisms underlying the effects observed in this study.

1094 - Characterization of Internal Teat Sealant Behavior Throughout the Dry and Post-Fresh Periods

Author: Michelle P. Buckley, Gustavo S. Silva, Tiago Tomazi, Brian Miller, Jenna Bayne, Jantijn M. Swinkels, Patrick J. Gorden

Objectives

The objective of this study was to characterize the behavior of two commercially available internal teat sealants (ITS) during the dry period as well as post-calving sealant shedding patterns in dairy cows. Parameters evaluated included milk leakage post dry-off, weight of sealant shed at first milking, duration of sealant shedding after calving, and amount of sealant shed at each subsequent milking. Udder health parameters evaluated included quarter-level intramammary infection (IMI) status at dry-off and post-calving as well as incidence of clinical mastitis (CM) in the first 120 days in milk (DIM).

Material and methods

Internal teat sealants evaluated included ShutOut® **(SO**, Merck Animal Health, Rahway, NJ, USA) and Orbeseal® (**ORB**, Zoetis, Parsippany, NJ, USA). All quarters of all cows received 500 mg of Cloxacillin Benzathine (Orbenin-DC™, Merck & Co, Inc., Rahway, NJ, USA). Thirty-two cows were enrolled, each treated with both sealants utilizing a cross-udder design. Teat pattern for each cow was randomly assigned the week before expected dry-off. Quarter-level milk samples were collected to evaluate udder health status using aseptic technique at dry-off and ≤14 days post-calving.

Sealant placement was visualized immediately after infusion using a TR9030 Generator and Next II Digital Radiograph machine (Sound Technologies, Carlsbad, CA). Imaging was repeated at 35-69 days dry. Milk leakage was monitored every eight hours for 15 minutes for three days following dry-off. After calving, the first 50 mL of milk from each quarter was collected and centrifuged. The supernatant was poured off and residue weighed to determine the amount of sealant present. At each subsequent milking, until unenrollment, individual quarters were hand-stripped ten times into a strip cup (Ambic Equipment Limited, Witney, UK) lined with a brown paper coffee filter to evaluate residue shedding post-calving.

Residue was scored on a 1-5 scale with 5 describing chunks of sealant >5 mm in diameter while a score of 1 indicated no sealant was present. Cows remained on this protocol until they achieved unenrollment criteria or were moved to the hospital pen. Unenrollment was achieved by meeting one of two criteria: (1) four consecutive milkings scoring $\underline{<}2$; or (2) six consecutive milkings scoring $\underline{<}3$.

Results

Based on radiographic images, there was no statistical difference between ORB and SO in cistern fill between treatments at dry-off (P=0.32) or pre-calving (P=0.41). At dry-off, 98.4% (124/126) of quarters had sealant present in the teat cistern (SO=98.4%; ORB=98.4%), while 97.6% (123/126) of streak canals had sealant present (SO=96.8%; ORB=98.4%). At dry-off, 21.1% (23/109) of teats had sealant present in the gland cistern (SO=23.6%; ORB=18.5%), which increased during the dry period to 55.9% (52/93

quarters) at pre-calving imaging (SO=59.6%; ORB=52.2%). Furthermore, 22.6% (26/115) of teats had no sealant in the cistern at calving (SO=25.9%; ORB=19.3%).

No statistical differences (P>0.05) between treatments were found regarding CM incidence (SO=7.8%; ORB=9.5%) and cured IMI (SO=93.3%; ORB=90.0%) and new IMI (SO=18.8%; ORB=17.9%). There was no significant difference in the milk leakage between groups and no correlation was detected between milk leakage after dry-off and IMI post-calving (P=0.67---), CM (P=0.26), or sealant weight at calving (P=0.67). No association was identified between pre-calving percent of cistern fill and post-calving infection status based on culture data (P=0.29).

Approximately 55% of quarters (SO=51%, ORB=58%) encompassing 90% of all cows had sealant present at colostrum collection. The presence of sealant decreased during the first ten DIM, however at day ten, 10% of cows had at least one quarter shedding sealant (score 4 or 5). Additionally, 11.5% of quarters had sealant flakes from 11-15 DIM while 3.5% of quarters shed sealant from 16-20 DIM. There was no statistical difference between milkings to unenrollment between treatments (P=0.88). Quarters in which sealant migrated into the gland shed noticeable amounts of sealant for twice as long (mean: 14.3 milkings) compared to quarters that did not have sealant in the gland (mean: 7.1, P <0.001).

Conclusions

No statistical differences were found between the two products regarding sealant behavior and udder health dynamics during the dry period and after calving. Sealant was shed twice as long from quarters, where it infiltrated into the gland during the dry period. This study demonstrates that infiltration of the gland with ITS occurs commonly during the dry period despite appropriate product administration at dry-off.

1100 - EFFECTS OF PROPHYLACTIC AMOXICILLIN AND GENTAMICIN COMBINATION ON RETENSION OF FETAL MEMBRANES, BODY TEMPERATURE AND MILK PRODUCTION IN DAIRY COWS DURING THE POSTPARTUM PERIOD

Author: Zafer Mecitoğlu , Mehmet Emin AKKAŞ

Objectives

The aim of the presented study is to determine the use of amoxicillin gentamicin combination administered prophylactically to all animals after birth; 1. The incidence of retention of fetal membranes (RFM), 2. Postpartum (pp) body temperature increase in the first 15 days, and 3. Its effect on the average milk yield in the first 60 days.

Material and methods

The presented study was conducted in a single farm with a capacity of approximately 2000 lactating cows, using a total of 106 multiparous cows in the pp period, with the same care and feeding conditions. After birth, 53 cows with an odd last digit of their ear number were included in the GMX group, while 53 cows with an even last digit of their ear number that gave birth in the same time period were included in the control (C) group. While the combination of amoxicillin+gentamicin [Gentamox®, Hipra SA, Girona, Spain] was administered intramuscularly to the cows in the GMX group at a dose of 40ml/animal for the first 3 days postpartum, no treatment was administered in the control group. Considering the 2-day milk withdrawal period, both animals in groups GMX and C were included in the dairy herd on the 5th day of the study. The animals were followed for 60 days after birth and RFM (failure to expel fetal membranes within 24 hours after parturition), average milk yield in the first 60 days, and body temperature (≥39.5°C) in the first 15 days. Sigmaplot 14 software (Systat Sofware, Inc., California, USA) was used for statistical analysis of the data. Distribution normality of the data was evaluated using the Shapiro-Wilk test. Chi square was used to compare number of the animals that suffered RFM between groups, and t-test was used to compare the averages of the day of body temperature increase and milk yield between groups. For all analyses, $P<0.05$ was considered statistically significant.

Results

Six animals from each group were removed from the herd within first 60 days postpartum. Therefore, data from these animals were not included in the study. At the end of the study, the average lactation numbers were determined as 2.4±0.6 and 2.6±0.6 in the GMX (n=47) and C (n=47) groups, respectively. Although it was not statistically significant (P = 0.14), the number of cows that suffer RFM was determined to be higher in the GMX group than in the C group (GMX = 21 cows, C = 14 cows). While 23 animals in the GMX group had high body temperatures in the first 15 days of PP, this number was 30 in the C group. It was observed that body temperatures in the GMX group increased in the later days of the postpartum period compared to the C group (8.08±0.52 days vs 6.50±0.63 days; P=0.06). It was determined that the milk yields in the first 60 days, which included milk yields between the 5th and 60th days pp, were significantly higher in the C group than in the GMX group (C = 43.6±1.38 liters, GMX = 39.6±1.39 liters; P = 0.04).

Conclusions

Based on the results of the presented study, it was determined that the combination of amoxicillin and gentamicin administered prophylactically after calving could delay the time when body temperature rises. On the other hand, the finding that the number of RFMs tended to be high in the study group may be related to gentamicin's reduction of myometrial contractility, as stated in previous studies. As a result, it was determined that the application of amoxicillin and gentamicin to all animals after birth did not have any positive effect, and it was determined that its use in farms was not appropriate due to its negative effects on milk yield and possibly the retention of fetal membranes.

1104 - SmartDCT: An easy way to identify dairy cows eligible to the selective dry-cow therapy

Author: Silvia BESCHI, Luc DUREL , Denis JARRIN, Sofie PIEPERS, Sarne DE VLIEGHER

Objectives

With the new European Regulation 2019/6 on Veterinary Medicines that came into force on 28 January 2022, the prophylactic use of antibiotics (AB) and blanket dry cow therapy (BDCT) in dairy herds is no longer authorized. In the meantime, several algorithms using composite cow SCC information derived from Dairy Herd Improvement (DHI) recordings have been proposed to distinguish infected from non-infected cows at dry-off. An elegant way for applying selective dry cow therapy (SDCT) while minimizing the negative consequences for the cow's future performances could be to adapt the composite cow SCC threshold not only by cow parity but also as a function of the herd-level udder health status. Based on the latter findings, the Smart DCT app (VIRBAC S.A., France) was recently developed and launched in the European market. The main objective of this study was to compare the expected reduction in antimicrobial use at dry-off using the Smart DCT approach combining algorithms proposed by researchers in The Netherlands, the United Kingdom, Belgium, New Zealand, and The United States.

Material and methods

The data set comprised a convenience sample of 104 Belgian and Dutch dairy herds. All herds participated in the local program DHI program. The average herd size was 164 lactating cows, ranging from 43 to 713. The average herd milk SCC (herd SCC) was 223,613 cells/ml and varied from 80,868 to 411,434 cells/ml. For the Smart DCT approach, the SCC threshold to distinguish infected from non-infected cows at dry-off depends on the estimated prevalence of cows with a composite SCC>200,000 cells/ml on the last test-day before dry-off. Therefore, the percentage of high composite SCC cows was calculated per test-day and herd. Of 6,935 lactating cows, 4- to 6-weekly composite SCC records were available from the lactation of enrolment starting at the first test day after calving until the last test day before dry-off. Unfortunately, no accurate clinical mastitis records were available and could therefore not be considered. The average number of cows per farm included in the data set was 67 and ranged from 10 to 251. Microsoft Excel was used to analyze the data descriptively.

Results

The average percentage of cows that could virtually have been dried off without AB, according to the different algorithms, varied between 37.0% and 67.8%. The lowest reduction in AB use at dry-off is expected to be achieved via the NZ (i.e. SCC primiparous: <120,000 cells/ml and multiparous cows: <150,000 cells/ml for all test-days in lactation of enrolment) and the NL (i.e. SCC primiparous: <150,000 cells/ml and multiparous cows: <50,000 cells/ml at last test-day before dry-off) approach, respectively. The Smart DCT and UK algorithm (i.e. SCC <200,000 cells/ml at the last three test-days before dry-off) are expected to allow for the highest reduction in AB use at dry-off. For the Smart DCT approach, the percentage of cows estimated to be dried off without AB increased with a decreasing prevalence of high SCC cows. However, caution is needed in interpreting the data as the Smart DCT app always recommends BDCT from a herd prevalence of high SCC cows >30%.

Conclusions

The Smart DCT algorithm is an elegant tool to support farm vets in determining the most optimal SCC threshold for distinguishing infected from non-infected cows as the herd health status is considered. The expected reduction in AB use when applying the Smart DCT approach will depend on the prevalence of high SCC cows and the herd size. Especially on small farms with suboptimal udder health, only a limited number of cows will be eligible to be dried off without AB.

1105 - Effect of ancillary anti-inflammatory treatment of mild clinical mastitis cases in dairy cows

Author: Hernan Bertotti, Pedro Rodriguez Fernandez, Martin Pol, Jantijn Swinkels , Guillermo Gargantini, Luciano Borda

Objectives

The study aimed to evaluate the impact of an ancillary nonsteroidal anti-inflammatory (NSAID) treatment along with an intramammary antimicrobial treatment on somatic cell count, rumination, and clinical outcomes in cases of mild clinical mastitis (CM).

Material and methods

A total of 207 cows with CM were enrolled from August to October 2022 in a dairy herd located in Santa Fe province, Argentina. Lactating cows with milker-defined CM as detected by fore-stripping 3 times a day, were included. The severity of CM was assessed daily by a veterinarian using a three-grade scale. Only cows with CM grade 1 (abnormal milk, no quarter inflammation, no systemic symptoms) were considered eligible for the study. The eligible cows were randomly assigned to two treatment groups. The Control Group (CG) received a two-day intramammary antimicrobial therapy with cephalexin/kanamycin (Ubrolexin®, Boehringer-Ingelheim), while the Study Group (SG) received the same intramammary treatment as CG, supplemented with a single dose of transdermal flunixin meglumine (Banamine® Transdermal, MSD Animal Health) at a dosage of 3.3 mg flunixin per kg body weight. Animals were monitored daily until clinical cure or retreatment (with another two-day treatment with the same product) ensued. Retreatment occurred if there was no observed clinical cure after the milk withdrawal period or if the disease severity increased during treatment. Monitoring of each animal's behavior such as rumination pattern was conducted using an automated behavior monitoring system (SenseHub™ Dairy, MSD Animal Health). Reproductive performance was evaluated for 150 days after CM. At onset of CM (Day 0), and 7 and 21 days thereafter, milk samples were taken after discarding the first three streaks of milk, to determine SCC of the affected quarter by an electronic counter. The samples were preserved with Bronopol and kept at 4 °C until transport to the laboratory.

Results

At enrollment, there were no differences between the CG (n=101) and SG (n=106) in terms of age or Days in Milk (DIM) (131 ± 82 vs. 131 ± 82 for CG vs. SG respectively) (all $P > 0.15$); and rumination rate (23 versus 22 animals in CG and SG, respectively (p=0.74). After initiation of treatments, no significant differences were observed in clinical cure at day 7 among groups with 30 (28.3%) and 23 (22.7%) of no cured cases in CG and SG, respectively (P=0.30). Retreatment rate did not differ among groups with 15 (14.8%) and 10 (9.4%) of retreated cases in CG and SG, respectively (P=0.26). The veterinarian defined a low rumination case when there was a decrease in daily rumination time of 100 minutes or more the day the CM was diagnosed. It was noted that animals in the Study Group (SG) with low rumination on average recovered to their pre-CM rumination baseline 1 day earlier compared to animals in the Control Group (CG) (1.7 days for SG vs 2.7 days for CG, P = 0.053). No differences were observed in Somatic Cell Count (SCC) of the affected quarters at day 0 (8,634,000 vs 9,268,000 SCC/mL for CG and SG, respectively) and at day 7 (2,064,000 vs 1,819,000 SCC/mL for CG and SG,

respectively). However, 21 days after CM detection, SCC of the affected quarters were lower in SG (558,000 SCC/mL) compared to CG (1,706,000 SCC/mL) (*P*=0.019).

Conclusions

Although, the use of additional NSAID treatment for mild CM did not affect clinical cure or the need for retreatment, SCC count at day 21 was found to be lower in SG group as compared to the CG group. Likewise, rumination rate recovered to the pre-CM rate 1 day faster in SG group as compared to CG. This suggests that the additional NSAID treatment for mild CM cases may help in restoring rumination rate to normalcy faster and reducing SCC.

1113 - Effect of flow-responsive pulsation on teat tissue conditions and milking characteristics in Holstein dairy cows

Author: Ajay Singh , Madeleine Spellman, Haritha Somula, Matthias Wieland

Objectives
The objective was to investigate the effect of flow-responsive pulsation (FRP) on teat tissue condition and milking performance of Holstein cows milked 3 times a day. We hypothesized that the application of FRP would augment the traditional premilking stimulation by the human hand for the establishment of cows' maximum milk ejection capacity leading to shorter milking duration, enhance peak milk flow rate, reduce bimodality of milk flow, reduce duration in low milk flow rate, and improve post-milking teat tissue condition.
Material and methods
The study was conducted at the Teaching Dairy Barn of the College of Veterinary Medicine, Cornell University (Ithaca, NY). A total of 156 lactating cows, milked thrice daily, were enrolled in a switch-back design for 35 d consisting of alternate 1-wk periods of conventional (CON) and FRP mode of milking. Premilking stimulation consisted of predipping, forestripping and wiping of teats and resulted in an average (mean ± SD) stimulation duration of 13 ± 3 s and a preparation lag time of 69 ± 17 s. In FRP mode, the milking started with a pulsation rate of 50 cycles/min and a ratio 30:70, till milk flow reached the set threshold of 0.5 kg/min upon attachment of the milking unit. Once the threshold was met (milk flow rate > 0.5 kg/min), the pulsation switched automatically to normal milking mode with a pulsation rate of 60 cycles/min and a ratio of 70:30 till the end of milking. During the CON periods, cows did not receive additional stimulation by means of the pulsation and were milked in normal milking mode from the beginning of milking. The evaluation of machine-induced short-term teat tissue changes was performed during session 2 of days 3 and 4 of each week by visual assessment postmilking. The effect of FRP on milking characteristics was evaluated based on the outcome variables milk yield (kg), milking duration (s), 2-min milk yield (kg), and peak milk flow rate (kg/min). The data were obtained through the farm management software program [DelPro, DeLaval International AB]. Bimodality of milk flow was assessed using the incremental milk flow data over the initial 2-min of milking. Statistical analyses were performed using SAS with the PROC MIXED and GLIMMIX procedure.
Results
The duration of low pulsation mode [mean ± SD; median (range); q1; q3] in FRP mode of milking was 16.5 ± 5.4; 16 (1–34); 14 and 18 s. The odds of short-term teat tissue changes were lower for cows in the FRP than for cows in the CON mode [odds ratio (95% CI) = 0.41 (0.31–0.55)]. We detected no differences in milk yield among groups. The least square means (95% CIs) of milk yield for cows in both the milking modes (FRP and CON) were 14.3 (13.8–14.7) kg. Cows subjected to FRP had higher milking durations, peak milk flow rates and spent longer durations in low milk flow but had lower 2 min milk yield as compared to the CON milking mode. The least square mean values (95% CI) in the FRP and CON were 272 (264–281) and 270 (262–278) s for milking duration; 5.8 (5.5–6.1) and 6.0 (5.7–6.3) kg for 2 min milk yield; 5.0 (4.8–5.1) and 4.9 (4.7–5.1) kg/min for peak milk flow rate, respectively. The FRP group had lower odds of bimodality than CON [odds ratio (95% CI) = 0.67 (0.61–0.74)].

Conclusions

The use of FRP in milking cows to supplement the manual premilking stimulation showed beneficial effects on teat condition such that the odds of machine milking-induced short-term teat tissue changes were lower compared with the CON mode. In addition, cows subjected to supplemental stimulation by means of the FRP had lower odds of bimodality, slightly longer milking unit-on time, and higher peak milk flow rate. The difference in milking duration was subtle with no practical implication. We conclude that using FRP to supplement manual premilking stimulation can facilitate a gentle milk harvest without compromising the milking performance.

1114 - The association of teat shape with bimodal milk flow in Holstein dairy cows

Author: Ajay Singh , Madeleine Spellman, Haritha Somula, Matthias Wieland

Objectives

The objective of this study was to investigate the association of teat shape with bimodality and incremental milk flow rates in Holstein dairy cows. We hypothesized that teat shape was associated with the occurrence of bimodality and the incremental milk flow rates.

Material and methods

A 31-d prospective cohort study was conducted using 3,697 lactating dairy cows from a commercial dairy farm near Ithaca, New York. Cows were milked 3 times daily at 8-h intervals in a 100-stall parallel rotary parlor. The rotational speed of the milking parlor was 4.9 s/stall. Premilking stimulation consisted of cleaning, forestripping, predipping and wiping which led to an approximate stimulation time of 6 s, and a preparation lag time of 93 s in early and 118 s in late lactation animals. The incremental milk flow rates (kg/min) included first 15 s milk flow rate (15S); 15−30 s milk flow rate (30S); 30−60 s milk flow rate (60S); and 60−120 s milk flow rate (120S) and were recorded with electronic on-farm milk meters and obtained through the adjunct software program [DelPro, DeLaval International AB]. Teat shape was assessed on day 1 of the study by visual assessment. Cows were categorized into 7 categories based on their teat shapes as follows. Cows with a uniform teat shape of all lactating quarters (excluding a nonlactating quarter, if present) were assigned as triangular barrel and pointed teat end (TP); square barrel and round teat end (SR); square barrel, round teat end and flat around the teat orifice (SRF); and square barrel and flat teat end (SF). For cows with all 4 lactating quarters and having 2 different categories of teat shape, 2 teats of each category were allocated to a combined teat shape (i.e., TP+SR, SR+SRF, SRF+SF). Bimodality of milk flow was assessed based on the incremental milk flow rates and recorded as a binary variable. Statistical analyses were done using generalized linear mixed model with ‘lme4’ and ‘nlme’ package in R.

Results

We analyzed 321,813 milking observations from 3,697 cows. Cows with flat teat ends had higher odds of bimodality than cows with a pointed or round teat-end shapes. The odds of bimodality increased from teat shapes TP to SF. The odds ratio (95% CI) ranged from 0.36 (0.20−0.64) in TP to 0.90 (0.52−1.56) in SRF+SF compared to SF. Lactation number was positively associated with the odds of bimodality. The odds ratios (95% CIs) were 0.26 (0.23−0.31) and 0.91 (0.78−1.07) for cows in the 1st and 2nd lactation number, respectively, compared to cows in lactation 3 and greater. Cows in late lactation had higher odds of bimodality than early and mid-lactation animals. Compared with late lactation cows, the odds ratios (95% CI) were 0.41 (0.35−0.49) and 0.48 (0.41−0.57) in early and mid-lactation cows, respectively. The incremental milk flow rates (15S and 120S) for cows in categories SR+SRF, SRF, SRF+SF, and SF were higher as compared to those in categories TP, TP+SR, and SR. The milk flow rates 30S and 60S were numerically higher in the cow groups with flat teats as compared to those with pointed or round teats.

Conclusions

The results of this study revealed that the teat shape is associated with bimodal milk flow in dairy cows. We attributed this relationship to the difference in milking speed across cows with different teat shapes. We conclude that in the presence of a fixed premilking stimulation regimen, bimodality is more likely to occur in cows with flat teat ends. Teat shape may serve as a useful phenotype to identify cows at risk of bimodality.

1115 - The effect of inclusion of a latency period in the premilking teat stimulation regimen, on teat tissue conditions and milking performance in Holstein dairy cows

Author: Ajay Singh , Madeleine Spellman, Haritha Somula, Matthias Wieland

Objectives
The objective of this study was to investigate the effect of inclusion of a latency period in the premilking udder preparation regimen, on milking machine-induced short-term teat tissue changes (STC) and milking performance of dairy cows milked thrice daily. We hypothesized that the application of a latency period resulting in a 90-s preparation lag time along with tactile stimulation would reduce the STC through augmenting the elicitation of the milk-ejection reflex. We further hypothesized that this would also lead to an increase of milk yield harvested, enhance milk flow rates, and reduce the milking duration.
Material and methods
This randomized controlled study was conducted at the Teaching Dairy Barn of the College of Veterinary Medicine, Cornell University (Ithaca, NY). A total of 145 lactating cows, milked thrice daily, were enrolled in a cross-over design for 14 d. The cows were randomly divided into 2 groups (TRT and CON) and assigned to one of the premilking udder preparation regimen for 7 d. After which, the cows were switched to the other udder preparation regimen for the next 7 d. The udder preparation regimen of the TRT group consisted of forestripping, wiping, and a latency period leading to a 90-s preparation lag time followed by attachment of the milking unit. The CON group udder preparation was like that of TRT except the latency period was omitted. This led to an average stimulation duration of 8 ± 2 s in group TRT and 9 ± 2 s in the CON group, and a preparation lag time of 88 ± 5 s and 14 ± 3 s in the TRT and CON groups, respectively. The evaluation of STC was performed during session 2 of days 5, 6 and 7 of each week by visual assessment postmilking. The effect of a latency period on milking characteristics was evaluated based on the outcome variables milk yield (kg), milking duration (s), 2-min milk yield (kg), and duration in low milk flow rate (s). The data were obtained through the farm management software program [DelPro, DeLaval International AB]. Bimodality of milk flow was assessed using the incremental milk flow data over the initial 2-min of milking and recorded as a binary variable. Generalized linear mixed models were performed using SAS with the PROC MIXED and GLIMMIX procedure.
Results
The odds of STC were lower for cows in the TRT group than for cows in the CON group [odds ratio (95% CI) = 0.13 (0.08–0.20)]. We detected differences in milking duration but no meaningful differences in milk yield among groups. Least squares mean values (95% CIs) for cows in the TRT and CON groups, respectively, were 246 (239–253) and 253 (247–260) s for milking duration and 15.4 (14.9–15.9) and 15.3 (14.8–15.8) kg for milk yield. The TRT cows had higher 2-min milk yield and they spent less duration in low milk flow rate. The durations of low milk flow rate in the 1st, 2nd and ≥ 3rd lactation animals, respectively, were 19 (17–22), 17 (15–20), and 13 (11–14) s in the TRT group and 31 (27–32), 22 (20–23), and 15 (13–18) s in the CON group. The TRT group cows had lower odds of bimodality as compared to the CON group across all lactations. Compared with CON

cows, the odds ratios (95% CI) for bimodality in the 1st, 2nd and ≥ 3rd lactation animals of TRT group were 0.19 (0.14–0.24), 0.29 (0.22–0.38), and 0.39 (0.28–0.53), respectively.

Conclusions

Cows that received a latency period between tactile stimulation and the attachment of the milking unit resulting in a 90-s preparation lag time had lower odds of STC, shorter milking duration, and spent less time in low milk flow rate to harvest the same amount of milk per milking session. We conclude that the inclusion of a latency period in premilking udder preparation regimens can help alleviating the negative effects of mechanical forces to the teat tissue during machine milking and has the potential to improve the milking performance.

1124 - Effectiveness of a Modified Live Vaccine for Vaccination of Dairy Bull Calves Against Mycoplasma bovis infection

Author: Douglas Hammon , Ken Mitchell, Dan Altena, Thomas Short

Objectives
To examine differences in mortality and health between calves that were, or were not, vaccinated with a commercially available modified live *Mycoplasma bovis* vaccine on a calf ranch.
Material and methods
This study was conducted on a commercial calf ranch in the western United States raising high-risk Holstein dairy bull calves with a history of *Mycoplasma bovis* disease from October of 2022 through March of 2023. Calves were usually 1-8 days of age upon arrival at the ranch. Two to four days after arrival 1,203 calves were randomly allocated to 1 of 2 treatment groups of approximately 150 calves per group each week for four consecutive weeks. The experimental group calves received a 2ml dose of a commercially available modified live *Mycoplasma bovis* vaccine (Protivity™) and control calves received an equivalent volume of saline. Calves were vaccinated for *M. bovis* at the time of enrollment and again 3 weeks later. All calves received a commercial intranasal IBR, PI3, BRSV vaccine (Inforce®3) upon arrival. All other vaccines were identical for both groups. All antimicrobial use in the first 35 days of life was limited to the beta-lactam family to limit any potential deleterious effect on the replication of a modified live *M. bovis* vaccine. Daily observations of health including morbidity, mortality and any abnormal health events were recorded through 120 days of age. Body weights were obtained at enrollment and at termination of the study. Using this data, total weight gains and average daily gains were computed for the period from arrival/enrollment through 120 days of age. The data were analyzed using mixed models for either continuous data (weight gain) or categorial data (health and mortality variables) using SAS 9.4 (SAS Institute, Cary, NC).
Results
There was no difference between groups in the incidence of reported health events including diarrhea, joint infections, respiratory disease, dehydration or umbilical infections. These were high risk calves and respiratory disease treatment rates in both groups were 100%. However, overall mortality was significantly less in the group that received the *Mycoplasma bovis* vaccine. Death loss in the control group (n = 603 calves) was 3.3 $\pm$ 0.8% in the first 5 weeks of life and 9.7 $\pm$ 1.4% from week 5 through the end of the 120-day study for an overall mortality of 12.5 $\pm$ 1.6%. In contrast, the vaccinated group (n = 600 calves) had a 1.6 $\pm$ 0.6% death loss in the first 5 weeks, 6.8 $\pm$ 1.2% from 5 weeks to 120 days and an overall study mortality rate of 8.3 $\pm$ 1.3%. That represents a 33.7% decrease (p=0.02) in death loss throughout the 120-day study period in calves receiving the *Mycoplasma bovis* vaccine. There was no difference in body weight at 120 days of age or average daily gain between groups.
Conclusions
The results of this study demonstrated that the use of a modified live *Mycoplasma bovis* vaccine during the pre-weaning period was able to significantly reduce overall mortality in high-risk dairy calves throughout the 120- day study.

1144 - EFFECT OF INTRAMAMMARY INFUSION OF PHAGES AND/OR ENDOLYSIN ON THE SOMATIC CELL COUNT AND MILK YIELD IN HEALTHY DAIRY COWS

Author: ABNER JOSUÉ GUTIÉRREZ CHÁVEZ, MARÍA ISABEL RUBIO-CORTÉS, JAQUELINA JULIA GUZMÁN-RODRÍGUEZ, DIANA RAMÍREZ-SÁENZ, JOSÉ ANTONIO HERNÁNDEZ-MARÍN, YOLANDA DANIELA CARRILLO-HUERTA, ALMA ARIANNA LECHUGA-ARANA

Objectives
The aim of the present study was to determine the effect of intramammary infusion of phages and/or endolysins on the somatic cell count and milk yield in healthy dairy cows.
Material and methods
After analyzing health and production individual records, 20 animals were selected to be included in this study. The criteria for selection were milk yield, Holstein breed, good udder conformation, apparent good health status and the absence of mastitis in any of the udder´s quarters. The final selection was complemented with support from the California Mastitis Test (CMT), somatic cell count (SCC) (DCC, DeLaval®) and microbiological test of milk by LB agar culture.
A total of 16 animals were randomly assigned to 3 experimental groups to receive a solution of bacteriophages (BK-510), endolysins, and a bacteriophage + endolysin solution. The fourth group was the control group with a saline solution. Milk samples were taken to monitor the response prior to the application of the corresponding treatment, at 0, 12, 24, 48, 72 and 96 hours.
The data was analyzed with a test of variance (ANDEVA) with a completely randomized factorial design and a significance of ($P<0.05$), using the StatGraphics® statistical package, a test of variance (ANDEVA) with a design of linear regression completely at random with a significance of ($P<0.05$), as well as a comparison of means using the Tukey method, comparing the treatments against the control using the Minitab 19® statistical package.
Results
All the mammary quarters included in the study were negative to the CMT and microbiological culture, which coincided with the low SCC ($<200X10^3$/mL).
Significant changes in milk secretion were recorded 8 h post-inoculation. It should be noted that, after 24 hours, an improvement in the quality of milk was observed, with the scarce presence of small lumps, which completely disappeared. Milk recovered a normal appearance after 72-96 h.
Cows of all groups recorded a slight decrease in the amount of milk produced. In relation to SCC, a significant increase was observed in the bacteriophage and bacteriophage with endolysin infusion groups, during the period between 8 and 24 h post-treatment. It should be noted that in the cows of the endolysin and control groups no significant increase in SCC was observed.
Regarding the monitoring of the presence of bacteriophages and endolysins in the milk samples, the observation of plaque-forming units (PFU) was recorded, showing no correlation with the type of treatment and/or the sampling time.
Conclusions
The intramammary application of a bacteriophage solution in healthy cows caused an increase in the number of somatic cells in the milk, which returned to its initial values

within 72 to 96 h post-treatment, while the application of endolysins did not show said reaction. The application of phages and/or enzybiotics represents an alternative therapy against resistant bacteria, with the advantage of being a sustainable tool for the control of mastitis, with little or no negative effect on the surrounding environment and even on the affected animal itself.

1146 - Bovine Ocular Squamous Cell Carcinoma. Epidemiological factors of Azores Islands Portugal

Author: Helena Maria Correia Vala, Fernando Esteves, João Rodrigo Mesquita, Rita Cruz, Carla Santos, Cristina Mega, Carmen Vasconcelos-Nóbrega, Dolores Fondevila, Maria Aires Pereira, Carlos Pinto

Objectives

High incidence of Bovine Ocular Squamous Cell Carcinoma (BOSCC) is recognized worldwide and, in Portugal, particularly in Azores islands (1), severely affecting health, the well-being of animals, causing low productivity, therapeutic and surgical costs and costs with partial or total rejections, in the case of multiple tumours.

The Azores islands are of volcanic origin, mountainous, with fertile soils, which easily retain water, creating green pastures that dominate the landscape and give them a privileged vocation for dairy farming, where the predominant breed is the Holstein-Friesian.

In the present work, a retrospective study was conducted focusing on the epidemiological and histopathological aspects of BOSCC in São Miguel on the island of São Miguel Azores.

Material and methods

A total of thirty-two tumours were collected at the moment of bovine slaughter, on São Miguel Island, Azores. The animals were not slaughtered or euthanized in order to carry out this study. Samples were obtained as sub-products derived from the normal activity associated with the meat inspection procedures. None of the actions was performed solely for research purposes and the researchers had no influence on slaughter organization nor in the meat inspection actions.

Samples were fixed in 10% neutral-buffered formalin and processed for routine histopathological diagnosis. All tumours were evaluated and classified according to World Health Organization (WHO) classification in Well Differentiated (WD), Moderately Differentiated (MD) and Poorly Differentiated (PD).

Results

All the animals diagnosed with BOSCC were Holstein Friesian breed with ages ranging from 4 to 10 years (mean 7.5 years). Nineteen samples were classified as BOSCC, from which eight had origin on the eyelids (4 WD, 2 MD, 2 PD), eight on the third eyelid (4 WD, 2 MD, 2 PD) and three involved the whole ocular region (1 WD, 1 MD, 1 PD). The remain thirteen samples (samples were classified as non-neoplastic lesions (ulcerative and/or inflammatory).

Conclusions

The predominant ocular localisation of the BOSCC studied was eyelids and third eyelid. No positive correlations were observed between location, age and degree of differentiation.

The extensive breeding regime in inclined slopes during all year, predisposes adult or old animals to high ultraviolet incidence, with the lighter areas of the face, including ocular region, being seriously affected in the development of neoplasms.

Acknowledgments: This work is supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the projects CITAB UIDB/04033/2020, CERNAS UIDB/00681/2020 and GHTM UID/04413/2020.

1148 - The effect of transport time on the first antimicrobial treatment on bull calves in one calf rearing unit

Author: Katja Mustonen , Marjukka Albrecht, Outi Jaatsi, Heli Simojoki

Objectives

In Finland dairy bull calves are typically transported at an early age from dairy farms either to specialized calf rearing farms or to fattening farms. Transport time is a known risk factor for increased morbidity in calves. Bovine Respiratory Disease (BRD) is one of the main health concerns in the calf rearing units causing increased use of antibiotics and high mortality in pre-weaned calves. It is often associated with transport stress. The aim of the study was to find out how transport time affects the time until the first antimicrobial treatment of the calves that were transported to a rearing unit.

Material and methods

The data was collected from transporting bull calves in seven different batches from multiple dairy farms to one rearing unit between January and August 2017. According to the national guidelines, only clinically healthy calves were accepted for transportation. In the vehicles, free access to water and bedding were offered. The rearing unit operated on an all-in all-out basis and the calves were transported to the finishing unit at average age of five months. The transport time from loading a calf at the dairy farm to unloading it at the rearing unit was recorded. The calves were examined by veterinarians one to three days after arrival to the rearing unit and BRD was diagnosed by the BRD scoring system (Sandelin et al., 2020). Antibiotic treatments administered at the rearing unit for each calf were recorded.

Statistical analysis was performed by using STATA/MP 17 for Windows (StataCorp LP, Texas, USA). A p- value < 0.05 was considered statistically significant. The batch was used as a random factor in a model. A linear mixed regression model was used to study the association between transport time and time to the first antimicrobial treatment. Vaccination status, breed, BRD diagnosis at the clinical examination and age and weight of a calf at the arrival were independent variables in the model used. The calves treated with antibiotics at the rearing unit were included in the linear regression model.

Results

Altogether 333 bull calves (174 AY, 159 HOL) were enrolled in the study. At the time of transportation to the rearing unit, they were 10 to 34 days old (mean age 17.0 days, median age 16.0 days). Transport time varied from 45 min to 13 h 25 min (average transport time 6 h 42 min, median transport time 6 h 38 min). 210 (105 AY, 105 HOL) calves were treated with antibiotics at least once during the study period. The time to the first antibiotic treatment varied from 0 days to 93 days (average time 21 days, median time 15 days). The longer transport time (hours) decreased the time to the first antibiotic treatment (days) (coef -0.942, p=0.044.). Other variables studied were not significant.

Conclusions

Though the transportation distances in Finland are moderate, the longer transport time decreased the time until the first antimicrobial treatment. The longer transport time might have effect on subclinical respiratory infections becoming clinical diseases.

References

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1151 - A Field study indicating that colostrum supplementation during the first 5 days of life reduces enteric pathogen shedding and respiratory disease in preweaned calves.

Author: Anna Catharina Berge , Geert Vertenten, Pleun Penterman, Iris Kolkman

Objectives

The objective of this study was to investigate the preweaning health, performance, immunity, and enteric pathogen shedding in calves supplemented with colostrum from dams vaccinated against rotavirus, coronavirus and *Escherichia coli* F5 during five days after birth compared to non-supplemented calves.

Material and methods

On a commercial dairy farm, healthy and viable new-born calves from vaccinated (Bovilis® Rotavec Corona) dams received at least 3 liters of colostrum from their own dam in the first 24h after birth and were randomly assigned to the colostrum supplementation treatment (treatment calves) or control treatment (control calves). The colostrum supplementation treatment consisted of the addition of a liter of previously frozen colostrum (from Bovilis® Rotavec Corona vaccinated cows) to the milk replacer on the second day of life and a half a liter of previously frozen colostrum to the milk replacer on day 3, 4 and 5 of life. Control calves similarly received once daily the same amount of supplement made from skimmed milk with similar protein and fat composition as the colostrum supplement. The daily milk intake and health parameters were monitored during the first month of life. Calves were weighed at birth, on day 28, and at weaning (approx. two months of age). Average daily gain (ADG) for the different periods was calculated. Faecal samples were taken at 7, 14, and 21 days of age. Additionally, on the first day of diarrhoeal disease diagnosis a faecal sample was collected. The presence of faecal pathogens was determined using a rapid ELISA kit (rotavirus, coronavirus, *Cryptosporidia*, *Clostridia*, *E. coli* F5). Faecal samples may also be evaluated for the microbiome composition. Serum samples were taken at 1, 7, 14, and 21 days of age to determine serum antibody levels to bovine coronavirus, rotavirus, and *E. coli* F5. Additionally, total serum IgG concentration was determined in the sample taken on day 1 to evaluate the transfer of passive immunity. Outcomes were analysed in multivariate statistical models controlling for calf age, passive transfer of immunity status, parity of dam and other potential confounders. In outcomes evaluating serial measures on calves, a repeated measures design of calf was used.

Results

Preliminary results are presented from 36 treatment calves and 34 control calves. The passive transfer of immunity in all calves was adequate to good and similar for both the treatment and the control calves. Limited enteric and respiratory disease was present. There was no significant difference in the diarrhoea hazard or the number of days to diarrhoea. There was significantly less respiratory disease in colostrum-supplemented calves. The ADG in the preweaning period of the calves was above 1 kg/day and no significant difference in ADG between treatment and control calves in the first month, the second month, or the overall preweaning period was observed. The faecal samples collected on day 7, 14, and 21 of age were all negative for coronavirus and *E. coli* F5. At 14 days of age, there was significantly less *Cryptosporidia* positive samples and a non-significant trend for less rotavirus positive samples in colostrum-supplemented calves

compared to control calves. The specific serum antibody levels against rotavirus, coronavirus, and *E. coli* F5 were not significantly different between treatment and control calves. Results from microbiome analysis is pending.

Conclusions

This study indicates significantly lower *Cryptosporidia* faecal shedding and a trend towards lower shedding of rotavirus in calves of 14 days of age in calves supplemented with colostrum from vaccinated dams the first 5 days of life compared to non-supplemented calves. Respiratory disease in calves under one month of age is significantly reduced in calves supplemented with colostrum the first five days of age.

1154 - Management of a digestive challenge in newborn calves thanks to a nutraceutical

Author: Frédérick Bussy NA, Audrey Chermat, Christian Engel, Preden De Cacqueray

Objectives

The objective of this trial was to evaluate the synergetic effect of an association between clay, wood charcoal and essential oils blend (named 'SafyGut Calf' or the product) on the gut health status of young calves.

Material and methods

Thetrialwasconducted on a dairy farm(200 PrimHolstein cows), in Brittany (France). Thefarmwas selected on veterinarian analysisconfirming the presence ofseveralrisk factors forcalfintestinal health, such ascryptosporidiosis, E.coli k99, rotavirusand coronavirus. Forty female calves were followed for two months frombirth. Calves were raised indoors in individual pens until weaning (done at two months old). The individual housing conditions remained identical during the trial for allcalves. Calves were alternatively allocated to the controland the test group, according to their date of birth. The test group received 10 ml ofthe product twice a day, from2 days old to 4 days old. The product formulated in an oral gel form, contains bentonite, wood charcoal and essential oils (thyme, clove, Chinese cinnamon and oregano). The same protocol was performed for the control group (the other half of the calves) with a placebo oral gel instead of the test product. The placebo gel comprises water, glyceroland glucose. During the trial, all veterinarian treatments were recorded daily for allcalves. Appetite, dehydration and feces status were evaluated at 6, 13 and 20 days old, by the same veterinarian fromChêne vert clinic (France), during the trial. Score were determined thanks to the scale described by Naciriet al. (1999) from0 (healthy calf) to 3 (poor condition of the calf). On the same days, cryptosporidiumpresence was detected on feces. Theanalyze, performed by areferencelaboratory (Labocea, France), isan oocystcounting (from50 to 300,000 oocysts) bymicroscopicreading after staining

Results

During the first week of life, calf feces consistency tended to be improved in the test group compared to the control group (Khi² p-value <0.1), with only 22% ofcalf presenting semi-liquid or liquid feces (diarrhealcalves) in the test group compared to almost 56% in the control group. The number ofcalves with dehydration score higher than 1 (persistence of skin fold) was lower in the test group (10% compared to 33%) even the difference was notsignificant. No difference was found for theappetitecriteria. During the second week of life, diarrhea severity has decreased almost fivefold in the test group (17% ofcalves with liquid feces in the test group compared to 83% in the control group, khi² p-value<0.1). These observations were corroborated by the number of calves receiving scour veterinary treatments (43%in thetest group compared to 67%in thecontrol group). During the trial, feces samples were collected on day 6, 13 and 20. In this farm, the peak ofcryptosporidiumoocysts shedding appeared around 13 days of life. However, it tended to reduce in the test group compared to the control group (Khi² p-value <0.1). The higher excretion in the control group was for 2 calvesat the maximumdetection level, meaning 300,000 oocytes. However, in thetest group, the maximumexcretionwas 17,000 oocytes. Theseresults showed that thecalves ofthetest group shed fewer oocysts.

Conclusions

Globally, test group calves had better feces consistency and less diarrhea treatments. Moreover, lower cryptosporidium oocysts shedding is noticed among calves fromthetest group. The product contains essential oils known for their antimicrobialeffect, whichmodulate the gut microflora (Gavaricet al., 2011). Associated with clay and wood charcoal, thesynergisticeffect oftheseingredients may reinforcethecalf digestivetract. Wecan assumethis formulationmay impair the cryptosporidiosis proliferation and its adverse effects on gut integrity. Further study is needed to understand the mode of action on the cryptosporidiosiscycle.

1156 - Ultrasound evaluation of mammary gland cistern in Holstein-Friesian cows affected by clinical mastitis

Author: Matteo Gianesella, Enrico Fiore, Anastasia Lisuzzo, Chiara Tommasoni

Objectives

Mastitis is one of the most important diseases in dairy cattle farms, which can affect the health status of the udder and the quantity and quality of milk yielded. The correct management of mastitis is based both on preventive and treatment action. With the increasing concern for antimicrobial resistance, it's strongly recommended to treat only the mammary quarters presenting intramammary infection, positive to antimicrobial culture. For this reason, a timely and accurate diagnosis is fundamental.

The possibility to detect and characterize mastitis directly on farm would be very useful to choose the correct management protocol. According to this, the objective of this study is to assess ultrasound mammary gland evaluation as a reliable on field diagnostic tool for clinical mastitis in dairy cows.

Material and methods

This study was carried out within the Agritech National Research Center. 45 mammary quarters of 23 Holstein Friesian dairy cows were evaluated. To avoid bias due to environment or management conditions, all animals were enrolled from a single dairy farm in the Veneto Region-Italy. Animals with antibiotic treatment prior the evaluation, or presenting other diseases differing from mastitis were excluded. Animal care and procedures were in accordance with the European directive 2010/63/EU and the national law D.L. 2014/26. During this study, sterile milk samples were examinated for antimicrobial analysis and somatic cell count (SCC), and clinical examination of all quarters and animals was performed. Based on the results, quarters were divided into 2 groups: "healthy" (20 quarters), having negative antimicrobial analysis and SCC< 100.000 cells/ml, and "sick" (25 quarters), having both positive microbiological culture and clinical signs of mastitis. After 6 hours from milking, B-mode mammary ultrasound evaluation was then performed. For this purpose, a portable ultrasound scanner equipped with a multi-frequency convex probe was used. Each quarter was longitudinally scanned in order to assess mammary health status, with a specific focus on the mammary cistern. Ultrasound images of the mammary cistern were then analyzed using using the ImageJ software. For each, mean gray value was obtained. Statistical analysis was performed through R software ver. 4.0.3 implemented with the 'rcmdr' package. The Shapiro-Wilk test showed no normal distribution of the data. The conversion to logartmic function was then applied in order to normalize mean gray values. Mean values and statistical difference between healthy and sick groups were finally evaluated through a One-Way ANOVA. P-value<0,05 was considered significant.

Results

From microbiological culture, eight animals were positive to coagulase-negative staphylococci (SCN), four to *Streptococcus uberis (S. uberis)*, two to *Escherichia coli (E. coli)*, one to *Bacillus* spp., one to coagulase-positive staphylococci (SCP), one to *Klebsiella*. Means with relative standard error of the mean gray values was calculated. Healthy quarters showed a mean value of 34,91±3, while sick quarters showed a mean value of 57,49±4,79. Considering the non-normal distribution of data, logaritmic conversion was applied. A significant difference of the logaritmic function of the mean

gray values between healthy and sick groups was found (p-value 0,0011). Respectively, healthy group showed a mean logaritmic value (and relative standard deviation) of 1,51±0,18; sick group showed a mean logaritmic value (and relative standard deviation) of 1,72±0,21.

Conclusions

Ultrasonography of the normal udder parenchima shows homogenous hypoechogenic parenchyma with interspersed anechoic blood vessel, milk alveoli and lactiferous duct and the gland cisterns appear as a large homogenous anechoic area with few hypoechoic dots corresponding to the milk. Several studies previously assessed ultrasonographic changes in parenchima structure and a possible grading based on them has already been proposed. Our study focused on the gland cistern instead. The statistical analysis highlighted a significantly higher mean gray values of quarters affected by clinical mastitis, indicating increased echogenity of the mammary cistern. In accordance with our results, many articles assessed the presence of echogenic content and of solid component in the gland cistern during mastitis. The possibility to predict and evaluate the degree of damage during mastitis through ultrasound mammary gland evaluation, particularly of the gland cistern, could represents an important on-farm tool. Moreover, the establishement of an objective mammary ultrasound score, based on mean gray values, might provide useful directions not only in terms of animal health and welfare, but also for the evaluation of the most proper therapeutic protocol.

1157 - Effects of adjunctive treatment with an injectable combination of butaphosphan and cyanocobalamin to individual antimicrobial BRD therapy on hematological and biochemical parameters in male Holstein-F

Author: Angelique Rijpert , Frédéric Vangroenweghe, Niels Geurts, Dominique Gevaert

Objectives

The Dutch white veal sector is considered highly specialized in raising calves. However, veal calves undergo serious challenges at a young age. Animals originate from different locations and experience several stressful events. This often results in high morbidity rates for bovine respiratory disease (BRD) and subsequent antimicrobial use. BRD impacts growth performance due to reduced appetite and high energy demands by the activated immune system. Fast recovery from a BRD infection is important to reduce the impact on performance and animal welfare. Adjunctive treatment in diseased animals with a combination of butaphosphan 100 mg/ml and cyanocobalamin 50 µg/ml (Catosal®, Elanco) improved the feed intake and recovery in dairy cows suffering from metabolic diseases.[1] Furthermore, increased antibody titers were found in calves receiving adjunctive treatment at vaccination.[2]

The study objective was to assess hematological and biochemical changes in male Holstein-Friesian white veal calves affected by BRD between 3 and 8 weeks of age, following adjunctive treatment with Catosal® for 3 consecutive days to a single injection of florfenicol (Nuflor®, MSD) (NCM) in comparison with calves receiving florfenicol only (N) in a Dutch commercial white veal farm.

Material and methods

In this blinded longitudinal cohort field study, 48 male Holstein-Friesian calves, were enrolled when their BRD score yielded ≥5 and ≤8 according to the score card modified from McGuirck.[3] Herd contained 1400 calves, originating from Germany, Luxemburg and the Netherlands with all-in all-out regime. Selected calves were randomly assigned to NCM (25) or N (23). From enrollment onwards, all calves received a single florfenicol injection of 40 mg/kg subcutaneously, additionally NCM received Catosal® 12 ml intramuscularly for 3 consecutive days. Serum blood samples were collected and BRD signs were scored at enrollment (SD0), at day 3 (SD3) and 8 (SD8) after enrollment. The panel included 15 biomarkers: albumin; urea; creatinine, calcium; phosphate; magnesium; creatinine phosphate kinase; alkaline phosphatase; aspartate transferase; gamma glutamyl transferase; glutamate dehydrogenase; bilirubin; non esterified fatty acids, beta hydroxy butyrate and haptoglobin. In addition, retreats after SD8 were recorded until slaughter. Statistical analysis was performed using JMP computational software (version 16, SAS Institute Inc.). Mean study day results and mean deltas between study day results of NCM were evaluated in comparison with N using one-way ANOVA and student T-tests. Significance was defined as $P < 0.05$, tendency for $P = 0.05$-0.10.

Results

At SD0, no significant differences were observed for any of the biomarkers nor for the clinical scores between NCM and N. Significant differences ($P<0.05$) were observed for magnesium at SD3 (0.76 and 0.71 mmol/L for NCM and N respectively). A tendency was observed for a higher calcium at SD8 in NCM versus N (2.58 and 2.51 mmol/L for NCM and N respectively) ($P=0.06$). The mean delta for calcium concentration SD8-SD3 and

SD8-SD0 differed between NCM and N (delta SD3-SD8: 0.07 and 0.003 mmol/L and delta SD8-SD0: 0.1 and 0.01 mmol/L for NCM and N respectively) (P<0.05). Creatinine decreased between SD3 and SD8 in NCM whereas the N group showed an increase. Also, the SD8-SD3 delta differed significantly (-3.64 and 2.96 µmol/L for NCM and N respectively) (P<0.05). For albumin and total protein NCM showed numerically higher concentration and less decline at SD3, but the difference was not significant. The average number of BRD retreats per calf for the rest of the fattening phase was 0.2 for NCM versus 0.35 in the N group.

Conclusions

The differences in evolution of calcium, magnesium and creatinine concentration in serum are indicative for a quicker recovery and higher feed and milk intake in NCM group versus N group. The metabolic differences were seen up to 6 days after the last treatment with Catosal®. Authors conclude that adjunctive therapy with Catosal® at the time of BRD infection helps to increase feed/milk intake and subsequent energy and nutrients supply. These are important factors to guarantee a quick recovery and lower the impact of BRD on performance and welfare.

1. Deniz, A., & Aksoy, K. (2022). Use of organic phosphorous butafosfan and vitamin B12 combination in transition dairy cows. Veterinární medicína.

2. Moraru et al.,2016, Change of blood morphophysiological parameters in calves during vaccination under influence of metabolism stimulator Catosal, proceedings World Buiatrics Congress Dublin, poster P02-002-151 p.431

3. Score card modified from S. McGuirck, University of Wisconsin-Madison

1160 - Ketoprofen as the sole initial treatment for mild and moderate bovine mastitis: efficacy and antibiotic reduction

Author: Volker Krömker, Ulrike Falkenberg, Nicole Wente, Yanchao Zhang, Franziska Preine, Julia Nitz, Anne Tellen, Stefanie Leimbach, Stéphane Trotebas, Juan Munoz-Bielsa, Philippe Gisbert

Objectives
Many farmers are not used to targeted mastitis treatment in conjunction with an on-farm test. Another possibility to use antibiotics more prudently in mastitis could be the initial treatment of all mild and moderate mastitis cases with an NSAID. Only if this treatment does not result in clinical cure within a desired time period, antibiotic treatment could be used.
Material and methods
In this clinical study carried out in three conventional northern German dairy farms between November 2022 and November 2023, all mild and moderate mastitis cases were randomly initially treated with either antibiotic (control group: C) or an NSAID (experimental group: E). Cows in group C received a 2-days intramammary treatment with cephalexin 200mg/kanamycin 100,000 I.U. one tube (Ubrolexin®, Boehringer Ingelheim) once daily). Cows in group E received three intra-muscular injections at 24 h intervals with 3 mg ketoprofen per kg bodyweight (Romefen PR 10% ®, Ketofen® 10%, Ceva Animal Health). If on the third day after the start of treatment with the NSAID, the clinical mastitis score had not improved by at least one level compared to the start of treatment, the animal was treated with intramammary antibiotics (as in the control group).
Results
A total of 217 clinical cases of mastitis occurred. The two groups did not differ in terms of lactation number, pathogen spectrum, lactation phase and mastitis history. In the experimental group, only 11.4% of mastitis cases needed to be treated with antibiotics. Clinical cure (CC) occurred significantly later in the experimental group (CC day 5: C: 83.9% vs. E: 58.3%) than in the control group. During the observation period, the groups did not differ in terms of recurrent mastitis cases (C: 4.2 % vs. E: 7.8 %) and new infections (C: 7.6 % vs. E: 5.3 %). The bacteriological cure rate was 79.8% in the control group and 60.9% in the experimental group ($p < 0.01$).
Conclusions
In the context of responsible antibiotic use the initial therapy with an NSAID such as ketoprofen in cases of mild and moderate mastitis is certainly a therapeutic option. The use of ketoprofen instead of antibiotics allows also for a lower quantity of discarded milk since it can be delivered again immediately after clinical cure (no withdrawal period for milk). It therefore reduces food waste and the costs of mastitis for the farmer.
The study also showed that the follow-up treatment with antibiotics is only necessary in a small proportion of cases.
Although, we found differences in clinical and bacteriological cure in favor of intramammary treatment, a targeted mastitis concept with pre-selection of cases to be treated with ketoprofen alone could avoid this limitation.

1163 - Effect of time of antimicrobial and anti-inflammatory therapy on the recovery process of feedlot cattle from an induced bovine respiratory disease model

Author: Diego Moya, Pedro Rodriguez Fernandez, Nathan Nathan Erickson, Emily Snyder, John Ellis, John Campbell, Samuel Wauer, Jason Nickell, Kiah Simpson

Objectives

The objective was to characterize the recovery process of beef calves treated 2 or 4 days after an induced bovine respiratory disease (BRD) model.

Material and methods

A total of 20 crossbred heifers (224.5 ± 25.70 kg BW) were exposed to a BRD induction protocol that used a viral/bacterial co-infection to replicate the pathogenesis in the field. Briefly, calves were loaded at the farm of origin into a sealed trailer (29 m^3 of air space) and exposed for 30 min to a virulent (in vivo passaged) dose of bovine respiratory syncytial virus (10^6 TCID50) aerosolized with an ultrasonic nebulizer. Calves were then transported for 25 min to the research facilities (Saskatoon, SK, Canada), where they were housed indoor in one pen with ad libitum access to grass and alfalfa hay bales and water. Sixty hours after the viral nebulization, calves received a 2-ml nasal dose of *Mannheimia haemolytica* (10^8 CFU), which determined the start of the trial (day 0). Calves were then homogenously assigned by body weight to one of the following two treatments (n=10): antimicrobial-NSAID treatment [Resflor®, Merck Animal Health, Kirkland, QC, Canada] containing Florfenicol (40 mg/kg body weight, BW) and Flunixin meglumine (2.2 mg/kg BW) via subcutaneous injection on d 2 (**2D**)]; or the same treatment but administered on d4 (**4D**). From d-2 to 47, heifers were observed daily by a veterinarian to evaluate the presence of clinical signs compatible with BRD. From d-2 to d 11, videos were recorded for continuous monitoring of cattle behaviors (eating, standing, and lying) by an observer blind to the treatments. On d-2, day of treatment, 11, 18, 32 and 47 of the trial, samples were collected to determine blood lactate, serum Amyloid A (SAA) and haptoglobin levels, white blood cell count, lung auscultation score and lung consolidation via ultrasonography, body temperature, and chute and exit temperament scores. On d4 and 47, arterial blood samples were collected to determine partial pressure of oxygen (pO2). Body weight was measured on d-3 and 47 to determine growth performance. The statistical analyses were done using the Mixed procedure for repeated measures from SAS [SAS OnDemand, SAS Institute Inc., Cary, NC, USA] including treatment, day and its interaction as fixed effects, and calf as the experimental unit.

Results

The clinical signs, predominantly coughing and nose secretions, started earlier ($P < 0.01$) and lasted longer ($P = 0.04$) in 2D than 4D calves. The 4D calves tended to spend more time ($P = 0.09$) lying down and to start fewer feeding events ($P = 0.06$) than 2D. There was a time x treatment interaction ($P = 0.04$) where the increase in the time standing up was greater in 2D than 4D calves from d 2 to 3 and from d 4 to 5. There was a time x treatment interaction ($P = 0.01$) where the reduction in the time eating was lower in 2D than 4D calves from d 1 to 2 and from d 7 to 8. There was a trend for a time x treatment interaction ($P = 0.09$) where haptoglobin was greater in 4D than 2D only immediately before receiving treatment. There was a time x treatment interaction ($P = 0.02$) where

the lung score was greater in 2D on d 11, but lower on d 47, than 4D. Lung consolidation was greater ($P < 0.01$) on d 11 than d 2, 4, 18 and 32 in both treatments. Both chute score and pO2 tended to be greater ($P = 0.08$) in 2D than 4D, while there was a time x treatment interaction ($P = 0.02$) where exit score was greater in 4D on d-3, but lower on d 47, than 2D. The 4D calves tended ($P = 0.06$) to have greater blood lactate levels than 2D.

Conclusions

The recovery of beef calves treated earlier (2D) with an antimicrobial-NSAID therapy was characterized by the presence of more clinical signs, but a healthier behavioral time budget allocation compared to those treated later (4D). The lower levels of haptoglobin and lactate, along with increased pO2 levels, indicates that 2D calves had reduced inflammation, lung distress, and enhanced oxygen supply compared to 4D. These findings suggest the importance of early intervention in the recovery process of calves treated for BRD.

1168 - Mastitis in dairy cows: influence of Streptococcus agalactiae and Prototheca spp. subclinical intramammary infection on blood metabolome

Author: Anastasia Lisuzzo, Luca Laghi, Enrico Fiore, Alessio Cecchinato, Vittoria Bisutti, Sara Pegolo, Diana Giannuzzi, Rossella Tessari, Antonio Barberio, Eliana Schiavon, Elisa Mazzotta, Chiara Tommasoni, Matteo Gianesella

Objectives

Mastitis is one of the most significant diseases in dairy cows and causes several economic losses. Once the mammary glands have been infected, the inflammatory response results in increased somatic cell count (SCC). For this reason, the SCC is often use as an indirect diagnostic tool for mastitis with a threshold of 200,000 cells/mL. *Streptococcus agalactiae* is a major cause of contagious subclinical mastitis worldwide causing an important influx of SCC from blood into milk. Conversely, *Prototheca spp.* is an alga inducing chronic granulomatous environmental mastitis, which is not always related with increased milk SCC. Subclinical mastitis could involve a potential systemic metabolic response that can be studied using the metabolomics approach. The aim of this study was to investigate the changes in the serum metabolomic profile upon subclinical intramammary infection (sIMI) from *Streptococcus agalactiae* and *Prototheca spp.* in Holstein dairy cows.

Material and methods

A cross-sectional study design was used and received an institutional approval by the Animal Care and Use Committee and by the Italian Ministry of Health (protocol number 510/2019-PR of 19/07/2019). All multiparous Holstein-Friesian dairy cows from a single dairy farm with more than 120 days in milk, absence of clinical signs of disease, and no antibiotic treatment or anti-inflammatory medications before enrollment were considered for the study. Mammary gland health status was assessed by bacteriological examination on individual milk (time-0) repeated two weeks later (time-1). Only animals with the bacteriological analysis confirmed at time-1 were selected. From those animals, a blood sampling was performed from the jugular vein at time-0 and used for serum metabolomics analysis by ^{1}H-NMR. Consequently, forty dairy cows that respected the above inclusion criteria were enrolled. Three groups were established based on bacteriological results: Control (n=16; negative); Agalactiae (n=17; sIMI caused by *Streptococcus agalactiae*); and Prototheca (n=7; sIMI caused by *Prototheca spp.*). The statistical differences of metabolite's concentration were assessed by linear model with the fixed effect of group and the SCC as covariate. A post hoc pairwise comparison among metabolite concentrations was performed using Bonferroni correction. A *p-value*<0.05 was accepted, whereas a 0.05≤*p-value*≤0.10 was considered as trend to significance. A hierarchical clustering heatmap was used to identify metabolites' cluster for the pathway analysis. This analysis was used to assess the metabolic pathways influenced by mammary health status using Fisher's exact test with Holm Bonferroni correction.

Results

The hierarchical clustering heatmap identified two main cluster, the second of which showed three subclusters. The sIMI groups showed a decreased protein synthesis related to phenylalanine, alanine, isoleucine, threonine and tyrosine, and an increased synthesis related to histidine, glycine, valine, and proline. These groups also showed a

lower function of phenylalanine and tyrosine biosynthesis and a greater metabolism of glycine, serine and threonine. Furthermore, the Agalactiae group had an increased protein synthesis related to glutamine, methionine, and leucine compared to Prototheca group. Forty-two metabolites were identified by metabolomic analysis, of which ten were significant and four tended to be significant. The sIMI groups showed higher concentration of histidine and lactose, and lower concentration of acetate, asparagine, and dimethylamine. The Prototheca group had higher values of 3-hydroxybutyrate and lower values of carnitine compared to the Control group. Moreover, the same group showed greater values of citrulline, and lower values of acetone, citrate, and ethanol compared to both Control and Agalactiae groups. The Agalactiae group had greater concentration of allantoin, and decreased values of methylguanidine compared to Prototheca group; and greater values of valine compared to the Control group.

Conclusions

Metabolomic analysis through ^{1}H-NMR is a useful tool to achieve knowledge about systemic response to subclinical mastitis in dairy cows. The metabolomic profile of our animals affected by sIMI are suggestive of a systemic involvement and that the metabolomic profile of animals undergoes changes related to the etiological agent of mastitis. The main alterations were related to ruminal fermentations, energy metabolism, urea synthesis and metabolism, immune and inflammatory response, and mammary gland permeability. This study demonstrates that these changes may be a risk factor for the incidence of recurrence and chronic mastitis, as well as a potential predisposing factor for secondary metabolic disorders under special conditions such as negative energy balance.

1170 - Current and recommended internal teat sealant application procedures. What we can we learn from dairy farmers in Northern Spain.

Author: C. Carbonell, L. Elvira, M. Marcos, J. M. Swinkels

Objectives

The European Union has introduced new regulations restricting the prophylactic use of antimicrobials in animal production (UE2019/6). As a result, Selective Dry Cow therapy (SDCT) is mandatory by law in Spain from January 2022.

Farmer's attitude is crucial to successfully implement SDCT, and to overcome their fear of reduced udder health status of the herd. Management and hygiene are also essential for the success of SDCT. However, in a recent study, we found a frequent underestimation of key aspects of the dry off procedure and a lack of knowledge about SDCT by Spanish dairy farmers. Furthermore, the awareness of Spanish dairy farmers towards best practices in the application of teat sealants (TS) at drying off is also unknown.

The objective of this study was to describe, analyze, and interpret: 1) the on-farm use of TS, the type of insertion, and 2) the farmer´s perception of a new syringe designed to facilitate partial insertion (PI) of the TS and: 3) the importance of PI and the ease of cap removal to allow for PI, in the TS application procedure.

Material and methods

Fifty-nine farmers from the north-west of Spain, included in different udder health services, were selected based on convenience by local milk quality focused veterinary consultants, to participate in this study between August 2022 and January 2023. Each farmer received a written protocol with best practices to implement a correct drying-off procedure and a box with 24 tubes of a recently marketed TS (ShutOut®, MSD Animal Health), to apply in 6 cows. Afterwards, the farmer responded to a questionnaire consisting of 12 closed questions distributed in three blocks: 1) description of the farm, 2) on farm TS use and TS application practices, and finally 3) the opinion on the new syringe handling, the importance and ease of removing the cap, and PI of the tube point into the teat canal. In the last block, pre-defined closed responses in a Likert-type scale were designed to categorize the importance.

Results

Dairy farms were representative of the local herd size distribution; 44.1% had 100-200 and 30.5% had 200-500 milking cows, and only one farm had more than 500 milking cows.

Results showed that a high percentage of farmers already used a TS, in all quarters of every (72.9%) or some (18.6%) of their cows at drying off which agrees with our previous study (Carbonell et al., 2022). Fifty percent of farmers applied TS with complete insertion (CI), while 42.5% used PI. Interestingly, 7.5% were unaware of the different options for TS insertion.

Regarding farmer´s perception of the new TS syringe, most respondents (79.7% of farmers) agreed that to allow for PI, partial cap removal of the tube point with one hand, was easy or very easy (a score of 4-5 on a 1-5 scale). In addition, 72.9% of farmers agreed that PI itself was easy or very easy, although more than half (58.5%) had not even previously used PI. In fact, almost all farmers (93%) applied the new TS syringe with PI,

following the recommendation of the protocol provided instead of their usual mode of application (87% of farmers who used to apply TS with CI switched to PI).
Related to awareness of best practices in the TS application procedure, on a 1-4 scale, most farmers (72.9%) scored 3-4 (strongly agreed or agreed) that the easy one-handed cap removal of the tube point facilitates handling and hygiene and reduces the risk of tip contamination. It is also highlighted that the majority strongly agreed (65%) or agreed (19%) that PI protects the teat canal better, reducing the risk of damage.

Conclusions

The group of farmers enrolled in this study was aware of the importance of good practices in TS application at drying off and appreciated the opportunity for partial insertion. On the other hand, there is probably an opportunity for veterinarians to include trainings about the drying off procedure in their udder health services.

1174 - Effects of generic tulathromycin compared to the pioneer tulathromycin on respiratory disease control, performance, and carcass characteristics in high-risk commercial feedlot cattle in the US.

Author: Shane Terrell, Jessica Newberry , James Lowe, Corbin Stevens, Kip Lukasiewicz, Nels Lindberg, Dan Thompson, Fabrice Payot

Objectives
The objective of this study was to evaluate the effect of generic vs pioneer tulathromycin for control of bovine respiratory disease.
Material and methods
This study was performed at a 30,000 head feedlot in western Kansas, United States. Heifers utilized in this study were at high risk of developing bovine respiratory disease (BRD) based on comingling, age, weight and transportation distance.
A randomized complete-block design with 14 replicates per treatment and one replicate per block (N = 28 pens; 2,451 heifers total) was utilized in this study, with the pen being the experimental unit. Source of the cattle and arrival date served as the blocking factor. During arrival processing for each block, cattle were randomly allocated to 1 of the 2 treatments. Animals in TUL treatment group received a subcutaneous (SC) injection of tulathromycin [Tulissin® 100 (tulathromycin injection)] in the neck region at a dosage of 2.5 mg/kg body weight (BW). Animals in the DRA treatment group received a SC injection of tulathromycin [Draxxin® (tulathromycin injection)] in the neck region at a dosage of 2.5 mg/kg BW. grade carcass outcomes were also compared.
Results
No significant differences (P≥ 0.05) were observed for any health variable. No differences were observed for average weight at enrollment, dry matter intake, or feed-to-gain ratio among groups. Slight differences exist within the deads and removals in analysis among average final weights and average daily gain; however, variation is likely due to numerical differences in mortality that were not significant. Although not statistically significant, separation in cumulative incidence curves representing total mortality, BRD mortality, total mortality, and total outs (mortalities + removals), respectively, between days 0-7 was also noted. Growth performance is estimated to be equivalent among both products. No significant differences (P≥ 0.05) were detected for hot carcass weight, dressing percentages, quality nor yield grades.
Conclusions
In this study, the effect of control of respiratory disease was evaluated by comparing administration of generic tulathromycin [Tulissin® 100 (tulathromycin injection)] to the pioneer tulathromycin [Draxxin® (tulathromycin injection)] on health, performance, and carcass outcomes. Based on the results of this study, evidence does not support a difference in health, performance nor carcass outcomes across TUL and DRA groups.

1175 - An Evaluation of a Generic Tulathromycin Injection in Diary-Breed Grower Calves at Ultra-High Risk of Developing Undifferentiated Fever/Bovine Respiratory Disease in Western Canada

Author: Calvin Booker, Jessica Newberry , Tye Perrett, Deborah Johnson, Sherry Hannon, Christopher McMullen, Fabrice Payot

Objectives
Evaluate the relative efficacy of tulathromycin [Tulissin® 100 (tulathromycin injection)] when administered to ultra-high risk neonatal Holstein and beef x dairy crossbred calves raised under commercial calf grower conditions in western Canada.
Material and methods
Candidate animals for the study were 7-to-14-day-old Holstein and beef x dairy crossbred bull and heifer calves that had been comingled from different dairies of origin at buying depots and transported considerable distances, thus making them at ultra-high risk of developing bovine respiratory disease (BRD). Calves arrived between 21-Sep-2021 and 28-Feb-2022, and were randomly allocated at the time of arrival processing. Animals in the **TULI** group (2381 animals) received a subcutaneous (SC) injection of tulathromycin [Tulissin® 100 (tulathromycin injection)] in the neck region at a dosage of 2.5 mg/kg (1.1 mL/100 lb) body weight (BW) at arrival processing. Animals in the **CTRL** group (2388 animals) received a SC injection in the neck region of tulathromycin [Draxxin® (tulathromycin injection)], at a dosage of 2.5 mg/kg (1.1 mL/100 lb) BW at arrival processing.
Study animals were housed in individual hutches until an average of ~35 days on feed. Calves were then moved to small pens for weaning and subsequently transferred to grower corrals. Calves from both experimental groups were commingled from the out-of-hutch event until shipment to the feedlot. The experimental unit was the individual animal. Animal health and production outcome variables were measured from allocation at the calf grower operation until arrival at the feedlot. Animals that did not die, were not chronic, and were weighed on arrival at the feedlot were included in the production analysis (**TULI** = 1879 animals; **CTRL** = 1881 animals).
Results
There were no differences detected in any of the morbidity or mortality variables between the experimental groups at the $P < 0.050$ level. This includes initial treatment for BRD, first BRD relapse, chronicity and wastage.
The experimental groups were considered homogenous ($P \geq 0.050$) with respect to average individual allocation weight. There were no differences detected in allocation weight, final weight, total weight gain, days on trial or average daily gain between the experimental groups at the $P < 0.050$ level.
Conclusions
No differences were observed between generic tulathromycin [Tulissin® 100] and pioneer tulathromycin [Draxxin®] in morbidity, mortality, or production parameters at the $P < 0.050$ level, suggesting comparable efficacy between the 2 antimicrobial products in neonatal dairy calves raised under commercial calf grower conditions.

1189 - Mycobacterium bovis wildlife surveillance in the Southeast – Isthmus Region of Mexico

Author: JOSE ALFREDO GUTIERREZ REYES, ALEJANDRO PERERA, SALVADOR DIAZ, ABNER JOSUE GUTIERREZ, ISAAC VELASCO

Objectives

The Bovine Tuberculosis (bTB) eradication program in Mexico establishes regions of cattle TB low prevalence. However, habitat reduction, the increase of intensive bovine production units, the increase of trade and mobilization of cattle among regions rises the possibility of contact between cattle and wildlife populations, representing a greater risk of exposure and transmission of bTB. The main objective of this work was to determine if *Mycobacterium bovis* (Mbovis) is present in the wildlife of localities where there are infected cattle herds based on activities of inspection for bTB of Between 2015 and 2022 in the states Veracriz and Chiapas in the south region of Mexico.

Material and methods

- Epi Study: Observational, Descriptive, Cross-sectional from FY 2016 to 2022 .
- Study area: South part of Veracruz and Central Chiapas States in the Southeast Region Isthmus of Mexico with 157,402 km^2
- Inclusion criteria collection: Specimens captured in a 1 km transect around Mbovis-infected cattle herds, confiscated and roadkill specimens, water buffalo herds under investigation for possible Mbovis infection.
- Diagnostic tests: Intradermal reaction, postmortem inspection, Histopathology & Bacterial culture for isolation.
- Sampling: 320 water buffalo and 5 spider monkey tested by PPD intradermoreaction and 24 specimen tissue sampled from different species of resident and transitory wildlife related with 6 infected bTB cattle herds.
- Analysis: XLSTAT Trial Software for Windows XP 9.3 was used for Statistic Data Analysis

Results

Active Epidemiological Surveillance: One granulomatous lesion in the trachebronchial nodule of a 96-month-old female water buffalo was identified in the regular slaughter at the "Mundo Nuevo" slaughterhouse in Coatzacoalcos, with compatible histopathological results and negative isolation; The Epi investigation included 2 herds of water buffalo with 320 head were tested by CFT with negative results. Likewise, during the study period, 5 spider monkey specimens confiscated by the authorities in the municipality of Palenque, captured in jungle areas invaded by cattle ranching, were negative for abdominal intradermal testing. Additionally, 12 samples of lymph nodes from specimens of different species like collared peccary, brocket deer, opposum, lowland paca, northern tamandua and whited nose coati were included as part of the epidemiological surveillance of this area with no ID of Mbovis infection, however the isolation of atypical mycobacteria was obtained

in 1 armadillo and 1 opossum not related to Mbovis-infected herds. Induced monitoring in wildlife: Related to 6 infected cattle herds from the municipalities of Sayula de Aleman, Pajapan, Hidalgotitlan, Oluta and Coatzacoalcos in southern Veracruz and 3 more in the municipalities of Pichucalco, Villaflores and Pijijiapan, samples of 11 specimens of Armadillo, Brocket deer and opossum were obtained without identify evidence of Mbovis. Statistical analysis by Chi square results in P = 0.294 and Wilks G^2 P = 0.176 (Observed value), respectively, which seems to indicate that the relationship between bTB in cattle herds is not related to wildlife of the region.

Conclusions

Considering the breadth of this region and the high number of herds and diversity of wildlife, these results should be interpreted as preliminary. What has been obtained so far contrasts with the results published in countries such as Spain, which may be due to the population densities in the areas under study, the bTB intra-herd prevalence and the customs of management or consumption of wildlife by the human populations in the zones that could affect the transmission of Mbovis between species. Therefore, it is required to continue with larger studies, including a greater number of herds, specimens and analyzes with the support of molecular epidemiology tools.

Meanwhile, it will be useful in the process of eradicating bTB in cattle in Mexico, the identification of risk factors and associated strategies to reduce the possibility of transmission from affected herds to wildlife or vice versa, considering:

- The update of regulatory framework for having a permanent official surveillance system in high risk areas.
- Implement a consistent database to adequately document the methodology and results of the sampling.
- A communication and awareness system for ranchers, hunters and related professionals on the subject on the timely reporting of possible cases of TB in wildlife.

1196 - Congenital Cleft Palate with other Orthopedic Malformations in a Korean Native Calf

Author: namsoo kim , Dongbin Lee, Suyoung Heo, kichang Lee

Objectives

Introduction: Congenital cleft Palate is a failure of the palatine midline fusion in mouth because of the delay in tongue descent during fetal development. Many cases of cleft palate occur in combination with other orthopedic malformations such as arthrogryposis, torticollis, scoliosis and spina bifida and most affected calves have inhalation pneumonia

Material and methods

Materials and Methods: 5 days old female Korean Native Calf was referred with arthrogryposis and cervical scoliosis. During physical examination, cleft palate was revealed and there were signs of severe pneumonia in lung auscultation. The fetlock joint of right forelimb affected with arthrogryposis and computed tomography(CT) was used to evaluate the degree of severity of arthrogryposis. Magnetic resonance imaging(MRI) and serum chemistry were used to rule out Akabane disease. Surgical approach was made in buccal lesion. The cleft extended from incisive papilla to the posterior border of the soft palate. It was approximately 20mm wide in the hard palate and 10~15mm wide in the soft palate. It was not enough to repair the hard palate both sliding bipedicle flap and overlapping flap technique. Therefore, bone graft used to reconstruct the hard palate. Half of the iliac wing incised longitudinally and attached by bone plate and screws in defected lesion. Sliding bipedicle flap technique performed overlapped the iliac wing. In the area of the soft palate, it was closed by combination with double layered flap and buccal flap.

Results

Results: Postoperative care included antibiotics and steroid to control inflammation. Partial and total parenteral nutrition supplied for maintain energy needs via jugular vein. After one week of surgery, patient died for uncontrolled inhalation pneumonia and low serum albumin level(less than 1.0g/dl).

Conclusions

Conclusions: Normally surgical repair of cleft palate is economically unfeasible and undesirable for breeding purposes. Although the prognosis is not good. In this case, surgical management of cleft palate cleft was occurred too widely and deeply. Moreover, inhalation pneumonia arose and became severely because of late detection of cleft palate. However, if patients are diagnosed as soon as possible after birth and effective surgery is achieved, the prognosis would be satisfied.

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1217 - Investigation of hematological values in feral cattle of the Galapagos Islands (Preliminary Data)

Author: Alegría Martínez, Ramiro Fernando Díaz Bolaños, Geovanny Sarigu, Rommel Lenin Vinueza

Objectives

Ecuador has not reported the presence of a Creole breed in the country. For several decades, a bovine biotype has been reported to originate from Isabela Island in the Galapagos Archipelago, which have been present for approximately 4 centuries. In the 16th century, the Spanish introduced these animals as part of their colonizing activity. There are not many studies on these animals so it is believed that they may be a native breed. It is possible that the feral cattle of Isabela Island in the Galapagos Archipelago are the first criollo breed in the country. To this end, as a first step, a comparison of hematological constants between feral and domestic animals from local herds was carried out.

Material and methods

A total of 9 cows *Bos taurus* were used in this study. Four were feral animals from Isabela Island and five were domestic animals from San Cristóbal Island in the Galapagos. Physiological constants such as heart frequency, respiratory frequency, and rectal temperature were evaluated in all animals. Blood samples were taken from coccygeal vein or artery, using a 10-ml Vacutainer^{TM} tube with (EDTA) and whithout anticoagulant and stored at 4°C until analysis in the laboratory. Blood levels of urea, creatinine, total proteins, albumin, globulins, amylase, lipase, alkaline phosphatase, ALT, AST, potassium, sodium, chlorine, calcium, phosphorus, ionic calcium and magnesium were performed. A complete blood count was also performed, including: hematocrit, hemoglobin, erythrocytes, VGM, CGMH, leukocytes, platelets, P proteins. The results obtained were analyzed statistically using the Shapiro-Wilk normality test, and the comparative t-student and Wilcoxon tests using the R statistical package. *p*-values <0.05 were statistically different.

Results

The animals presented physiological constants within the normal parameters of the Bos taurus species. Heart frequency 81+/- 1.41 and 62 +/- 9.16; respiratory frequency: 36.26 +/- 9.53 and 36.33 +/- 9.81; and rectal temperature: 38.38 +/- 0.19 and 38.3 +/- 0.13 for ferals and domestic animals respectively. With respect to blood chemistry, the results were also within the normal physiological parameters for the species. However, it was observed that in AST, calcium, ionic calcium and magnesium, feral cows presented higher levels ($p< 0.05$) than domestic cows. On the other hand, no statistical differences were observed between the hemogram values of feral vs. domestic animals ($p>0.05$). These values were also found to be within the normal parameters for this species.

Conclusions

These results demonstrate the high capacity for adaptability that the feral animals of the Galapagos Islands because their physiological constants and hematological values have not changed despite all the time they have been exposed to the environmental conditions of the Galapagos Archipelago.

1224 - The relationship between the number of vaccination years and antibody titer in Holstein Dairy Herds Following Multi-Year Immunization with Mastitis Multivalent Inactivated Vaccine

Author: Tastuya Ando , Shiori Saito, Chihiro Kanazawa, Nodoka Komori, Tomoyuki Arima, Yoshiko Yamazaki

Objectives
This study was conducted to investigate the trends of antibody titers against Escherichia coli J5 and Staphylococcus aureus anti-slime in a Holstein dairy herd vaccinated with STARTVAC (HIPRA, SPAIN) since July 2018. The stability of antibody titers at calving and third vaccination, and the relationship between the number of STARTVAC vaccination years and antibody titers were evaluated.
Material and methods
The one dairy herd in Ishikari District, Hokkaido with 130 cows as of September 2023 was selected in this study. STARTVAC vaccination of the herd began in June 2018 using label protocol. Blood was collected at 45 days(1) and 10 days prepartum(2), at delivery(3) and 52 days postpartum (4) regardless to vaccination. Serum was cryopreserved and antibody titers were measured, and sera collected up to January 2023 were used for this assay. The antibody titers were evaluated as IRPC values, and the software "R" was used for statistics to examine the significance of each value at $p<0.05$.
Study 1: E. coli-J5 antibody (EC-AB) and SA anti-slime antibody (SA-AB) were evaluated within the same parturition group and with increasing the number of parturitions only for the animals vaccinated from heifer.
Study 2: EC-J5 antibody were evaluated within the same parturition group and with increasing the number of parturitions for the entire herd. The highest IRPC values were divided into four tiers by quartile analysis: $150 \leq (1)$, $125 \leq (2) < 150$, $100 \leq (3) < 125$ and $(4) < 100$, and the percentage and number of bacteria detected, number of days of postpartum examination and mastitis score were compared, respectively.
Results
Study 1: In EC-AB, antibody titer at 45 days at prepartum(E1) and 52 days postpartum(E4) were significantly higher in the same parturition group until the fourth parturition, and antibody titer at delivery(E3) was significant higher up to third parturition. A significant increase in E1 was observed with the increase in the number of parturitions up to third, after which there was no significant difference in E1. In SA-AB, antibody titer at 45 days at prepartum(S1) and 52 days postpartum(S4) showed significantly higher in the same parturition group up to second parturition. A significant increase in S1 antibody titer was observed up to forth parturition.
Study 2: The incidence rate of E. coli mastitis was decreased with increasing number of vaccination years, and the number of tests performed within 50 days postpartum decreased. In IRPC tier (1), there was a trend toward improvement in the incidence rate of E. coli mastitis, the number of bacteria, and the mastitis score, and the number of tests performed within 200 days postpartum decreased. Although the IRPC titer increased with the number of vaccination years, the relationship was not generally correlated, and a wide range of IRPC titers was observed.
Conclusions

The IRPC values increased with the number of STARTVAC vaccination years, but EC-AB and SA-AB showed different trend. The IRPC titers of both EC-AB and SA-AB increased with each successive year of vaccination, indicating that multi-year STARTVAC vaccination was effective. Changes were observed in the proportion of causative strains of mastitis, number of bacteria, number of days of postpartum examination, and mastitis score as the number of STARTVAC vaccination years and antibody titer increased. However, there were individual differences in the increase in IRPC titers due to the number of vaccination years, and although multi-year vaccination increased IRPC titers in the herd as a whole, the effect of continued multi-year STARTVAC vaccination in preventing mastitis and reducing symptoms was not due to the number of vaccination years, but mainly due to the increase in antibody titer.

1232 - ASSESSMENT OF THE EFFECT OF VACCINATION WITH A MULTIVALENT MARKER VACCINE ON IMMEDIATE MILK PRODUCTION

Author: Mariona Tapiolas Verdera, Marta Gibert Lleixa, Marc Vila Poch, Marc Tomàs Sánchez, Ainhoa Puig Ambrós, Joaquim Mallorquí Bagué, Ricard March Massós

Objectives

Safety studies need to be conducted to verify the safety of new vaccines under field conditions. A multivalent viral vaccine developed to immunize cattle against bovine respiratory syncytial virus (BRSV), bovine herpesvirus type 1(BoHV-1), bovine viral diarrhoea virus type 1 and 2 (BVDV-1 and BVDV-2) and parainfluenza-3 virus (PI-3)(DIVENCE PENTA, HIPRA) has been tested after each dose administration in lactating cows according to the recommended use (EMA/CVMP/IWP/260956/2021).

In dairy herds, milk production is one of the most important parameters to determine possible adverse events of vaccination. A field trial was conducted with the objective of evaluating the effect of vaccination with DIVENCE PENTA on the milk production of dairy cows during the consecutive days after each vaccination dose (1st, 2nd and 3rd dose).

Material and methods

Two commercial dairy cattle farms (80 and 270 lactating dairy cows .) located in Spain with standard management conditions were included in the study.

Approximately 50% of animals from each farm were injected intramuscularly (2ml/dose) with DIVENCE PENTA (DIVENCE group) and the remaining animals with PBS (Phosphate Buffered Saline, Control group). The vaccination schedule consisted of two doses at an interval of 21 days apart, and a third administration approximately 6 months after.

On each farm, the 40 lactating cows with the highest milk production at the time of each vaccination (20 control and 20 vaccinees) were closely monitored. Individual milk production from these animals was electronically recorded from two days before vaccination to 14 days after, in order to evaluate a possible impact of vaccination on the milk production.

The statistical analysis was done using the Rstudio v3.1. A *p-value* < 0.05 was considered as the limit of statistical significance. Daily milk production comparison between groups was analysed by using the Kruskal-Wallis test .

Results

The evolution of mean daily milk production per group after each dose administration showed a very similar production curve between groups after 1st, 2nd or 3rd vaccination dose.

No statistically significant differences were observed between groups in mean post-vaccinal milk production after any vaccination dose.

In both DIVENCE and Control group, no statistically significant differences were observed between the two days before vaccination and one week after vaccination in any of the three vaccination doses.

Conclusions

The use of DIVENCE PENTA has demonstrated to be safe and not to reduce milk production in dairy cows after the administration of several doses across the lactation period. Milk production was not impaired by the use of the vaccine as compared to control animals.

1237 - PASSIVE IMMUNITY IN CALVES BORN TO A NEW MULTIVALENT-MARKER VACCINE INJECTED DAMS

Author: Marta Gibert Lleixa, Sara Baila Martínez, Marc Vila Poch, Mariona Tapiolas Verdera, Ainhoa Puig Ambrós, Ricard March Massós, Joaquim Mallorquí Bagué

Objectives

Newborn calves are immunologically naive at birth. The ingestion of colostrum is essential for providing neonates with immunologic protection during at least the first 2 to 4 weeks of life (Chase et al., 2008). Nevertheless, IBR colostral antibodies persist for 4-6 months (Petrini et al., 2019), while BVD antibodies may persist for 6-11 months (Chamorro et a., 2014). A proper approach to achieve passive immunity in newborn calves is through vaccination of the dam.

The present study aims to evaluate the effect of maternal immunization against BVD and IBR with DIVENCE PENTA (HIPRA, Spain) vaccine on the transfer of passive immunity to calves.

Material and methods

Cows and heifers at different pregnancy stages from two dairy cattle herds were included in the study. Animals were randomly distributed in two groups stratified according to pregnancy stage. One group was vaccinated intramuscularly (2ml/dose) with the DIVENCE PENTA vaccine (DIVENCE group), while the other group was administered intramuscularly (2ml/dose) a placebo (Phosphate Buffered Saline, PBS-Control group).

All animals received the basic vaccination schedule (two doses of the corresponding study product 21 days apart) prior to calving. Animals at the inclusion day (day 0, first dose vaccination) found in the first trimester of pregnancy, received an additional dose 6 months after, making a total of three doses before calving.

The immunological response was assessed at calving. For that purpose, colostrum and serum samples from the dams were collected within 24 hours of calving. Serum samples from the newborns were collected at birth (between 2 and 7 days after birth; D2/7), after colostrum feeding of their own dam, and one month later (D30).

Colostrum and serum samples were analyzed for BVDV and BoHV-1 antibody detection by means of ELISA (IDEXX BVDV Total Ab and CIVTEST®BOVIS IBRgB for serum/CIVTEST®BOVIS IBR for colostrum). Newborn serum samples were also analyzed for neutralizing antibodies detection against BVDV-1, BVDV-2 and BoHV-1 by virus neutralization test.

The statistical analysis was done using R software v4.3.1. A p-value < 0.05 was considered as the limit of statistical significance. Comparison between groups was performed by the nonparametric Wilcoxon Mann-Whitney test.

Results

Samples from 37 animals in the PBS-Control group and 36 animals in DIVENCE group were collected.

Colostrum samples from DIVENCE group presented a significantly higher mean antibody titer both for BVDV and BoHV-1 compared to PBS-Control group. Serum BVDV ELISA

antibody titers from the DIVENCE group were significantly higher in comparison to PBS-Control group. Serum samples from the PBS-Control group remained seronegative and below the cut-off value for all samplings. Newborns from the DIVENCE group presented greater values than dam serum samples. Specifically, the highest titers were observed at birth timepoint (D2/7), while remaining significantly high one month after (D30).
In the same line, the antibody response against IBR was statistically significantly higher in the DIVENCE group compared to the Control group for all sampling timepoints. Neutralizing antibodies observed in newborn animals of DIVENCE group against BVDV-1, BVDV-2 and IBR were statistically significantly higher than the PBS-Control group, both at birth and at D30 timepoints.

Conclusions

DIVENCE PENTA induces a significantly high antibody response against BVD and IBR in vaccinated dams at different pregnancy stages, which provides passive immunity to the newborn calves fed with colostrum from vaccinated dams.

1242 - Quantifying biosecurity in dairy cattle farms

Author: Laura Courtens , Catharina Berge, Nele Caekebeke, Ilias Chantziaras, Jeroen Dewulf

Objectives

By using the BioCheck.Ugent biosecurity scoring tool, the level of biosecurity in dairy farms can be assessed and quantified. Consequently, strengths and weaknesses are identified and can be addressed where necessary. It is available at https://biocheckgent.com and is free for use to anybody.

Material and methods

The biosecurity assessment is based on a questionnaire evaluating all the important external and internal biosecurity measures. The answers on the questions are translated into a risk-based score by means of an algorithm. The system provides a total biosecurity score as well as separate scores for external and internal biosecurity and each of the subcategories. Each of the scores varies between 0 and 100 with 0 meaning absence of any measure and 100 meaning a perfect application of the biosecurity measures included in the scoring system. The obtained score is benchmarked against the data of other farms available in the database.

Results

To date (Nov 2023), over 2000 BioCheck.Ugent surveys have been submitted worldwide from dairy cattle farms in Africa, Asia, Europe, Oceania, North America and South America. Dairy cattle farms score on average 66% (SD: 17, 6-100) on external biosecurity and 38% (SD: 17, 0-100) on internal biosecurity, resulting in a total biosecurity score of 52% (SD: 14, 3-100). For external biosecurity, the subcategories: 'Purchase and reproduction', 'Transport and carcass removal', 'Feed and water', 'Visitors and farmworkers' and 'Vermin control on other animals' score respectively: 77% (SD: 32), 47% (SD: 22), 58% (SD: 28), 68% (SD: 23) and 62% (SD: 23). For internal biosecurity, the subcategory averages were: 'Health management' scoring 32% (SD: 24), 'Calving management' 32% (SD: 19), 'Calf management' 44,% (SD: 21), 'Dairy management' 47% (SD: 18), 'Adult cattle management' 41% (SD: 27) and 'Working organisation and equipment' 39% (SD: 24). Global external biosecurity measures score higher compared to the global internal biosecurity measures indicating that more attention is paid to measures to keep pathogenic agent out of the farm compared to measures to prevent infection spread within the farm. It has been observed before that it may be harder to apply internal biosecurity measures as it requires changes and discipline in the daily work of the farmer whereas external biosecurity measures are more influenced by infrastructural measures and rules that can be applied upon others (e.g. visitors, truckdrivers,…).

National average scores are calculated when at least 40 data registrations are available for a specific country. National averages are currently available for Portugal, Finland, Belgium, Kyrgyzstan, North Macedonia, India and Kosovo. The overall highest scoring countries are situated in the European Union, where 'The EU Animal Health Law' legislation is implemented. The main focus of this legislation is on transmissible animal diseases and how to prevent and eradicate diseases in the livestock sector. There are several countries that have started to use BioCheck.Ugent in regulatory or obligatory health programmes.

Conclusions

The BioCheck.Ugent scoring system can evaluate the biosecurity level and evolution in time both within continents, countries, regions and farms. Thereby, the scoring tool can be used as a tool for supporting farmers towards a better biosecurity. This system also allows to study the association between biosecurity and factors such as production characteristics, antibiotic use and animal welfare. Biocheck.Ugent for dairy farms is currently also used to evaluate farm level biosecurity in relation to the prevalence of Bovine Coronavirus (also presented at WBC 2024 by Berge et. Al). The overall goal of the BioCheck.Ugent tool is to help to improve biosecurity worldwide.

1244 - Field experience: Efficacy of 2 vaccines for the control of Streptococcus Uberis, Staphylococcus and coliform mastitis and their consequences in a context of 23 farms with robotic milking

Author: Loic Bernard , Lorena Nodar, Richard Février, Elodie Dumas

Objectives

Robotic milking offers standardization of milking hygiene and data collection. This means hygiene of milking does not change over time. The data provided related to milk quality is solid and continuous , that is why the effectiveness of a multivalent vaccine against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) [STARTVAC®, HIPRA], and a monovalent vaccine against *Streptococcus uberis* (*S. uberis*) [UBAC®, HIPRA] was analyzed in 23 robotic dairy farms in France.

Material and methods

23 robotic farms bringing together 2.242 lactating cows at the start of the study (average 98 cows/farm) were vaccinated using 2 mastitis vaccines [STARTVAC® and UBAC®] together. Before implementing vaccination, a preparatory visit was carried out to provide updates on animal husbandry, robotic milking hygiene, and culling policy. Depending on the specificities of each farm and under veterinary control, the adopted vaccination protocol was as follows: Primary vaccination 3 injections of each vaccine spaced each 6 weeks apart on all lactating cows, dry cows and heifers to calve in the following 6 months. A booster vaccination is applied every 3 months for the *S. aureus* / *E. coli* vaccine [STARTVAC®] and every 6 months for the *S. uberis* vaccine [UBAC®]. The primiparous arriving next are included in the herd vaccination schedule after having finished their primary vaccination.

The start of vaccination corresponds to the 1st injection and the individual data collected (per farm) is daily. Four groups were compared: One year before vaccination (no vaccines/prevaccination), 0-6 months of vaccination, 6-12 months of vaccination and finally 12-24 months of vaccination. Average herd age was tested with a LRM (linear regression model) with farm as random effect. Somatic cell count (SCC) and discarded milk were analyzed through LRM with average age and season as covariables and farm as random effect. Also, on a monthly basis, clinical mastitis cases are recorded (only 18 farms carried out this reading) and analyzed through a Poisson regression with farm as random effect. The pre-vaccination period was between -408 days and -729 days. We want to indicate that the 23 farms had started up their robots more than a year earlier. The age of each cow was registered in order to follow the age evolution of the herd.

Results

Age

The average age is not significantly changing over the different time periods (p-val: 0.168).

Somatic cell count (SCC)

Significant differences (p-val < 0.001) of SCC between groups were found:

- Prevaccination198.000 cells/ml (c)
- 0-6 months 166.000 cells/ml (b)
- 6-12 months 139.000 cells/ml (a)

- 12-24 months 142.000 cells/ml (a)

The SCC already reduces in the first semester, and the difference is higher in the second semester as well as in the second year.

Discarded Milk

Significant differences (p-val < 0.001) of discarded milk between groups were found:

- Prevaccination 1,40 l/cow/day (d)
- 0-6 months 1,15 l/cow/day (c)
- 6-12 months 1,00 l/cow/day (a)
- 12-24 months 1,02 l/cow/day (b)

Clinical Mastitis cases (CM)

Significant differences (p-val <0.001) of clinical mastitis per cow and year between groups were found:

- Prevaccination 1,07 CM/cow/year (c) and 52,92% of cows with CM (c)
- 0-12 months 0,7 CM//cow/year (b) and 41,25% of cows with CM (b)
- 12-24 months 0,47 CM/cow/year (a) and 29,94% of cows with CM (a)

Significant differences (p-val <0.001) between groups were found of clinical mastitis cases per cow with clinical mastitis:

- Prevaccination 1,95 CM/cow with mastitis (b)
- 0-12 months, 1,65 CM/cow with mastitis (a)
- 12-24 months, 1,54 CM/cow with mastitis (a)

Both parameters already reduce in the first year, and the difference is higher in the second year.

Conclusions

This study confirms the preventive value of vaccination against mastitis as an aid to control the SCC and to reduce clinical mastitis cases. The robot environment provides us with the security that milking hygiene in all periods is the same and data could be gathered during the entire study. The statistically significant improvements are greater during the second comparative analysis period 12-24 months after the start of double mastitis vaccination [STARTVAC® and UBAC®].

1245 - Producers' and veterinarians' perceptions regarding the impact of frequent diseases or syndromes on calf health: toward dairy calf disability weight determination'

Author: Jean Silva Ramos, Marianne Villettaz Robichaud, Jocelyn Dubuc, Débora Santschi, Jean-Philippe Roy, Gilles Fecteau, Sébastien Buczinski

Objectives

In human science, the population's perception is used to understand the impact of a disease on individual health. In dairy science, only one study estimated this perception for frequent diseases in cattle and used it to develop a cow health summary measure.

The objective of this study was to assess and compare the perceptions of dairy producers and veterinarians regarding the impact of frequent diseases or syndromes on the health of pre-weaned dairy calves.

Material and methods

This project was approved by the local committee for research ethics in sciences and health (Protocol number 2023-4118). A questionnaire was conducted in person to assess the perceptions of 40 dairy producers (clients of the bovine ambulatory clinic of the Faculté de médecine vétérinaire de l'Université de Montréal, Saint-Hyacinthe, Québec, Canada), and 53 Québec veterinarians were contacted via email through their association to fill out the online version of the questionnaire. The questionnaire consisted of questions regarding demographic aspects of the respondent (age, sex, and years of experience) and calf health perception. A visual scale was employed to estimate the impact of each disease or syndrome on calf health (also known as disability weight). This scale ranged from 0 (minimal impact) to 10 (maximum impact, corresponding to a state of health that requires euthanasia or resulting in the death of the animal). Each participant was invited to provide their perception of the minimum, probable, and maximum impact of each disease or syndrome. A total of nine frequent diseases or syndromes related to calves were selected: diarrhea, dystocia, inadequate passive immunity transfer, fracture, presence of a wound or abscess, septic arthritis, respiratory disease, umbilical infection, and congenital defects. Data analyses were performed using R software. An overall distribution of opinions was developed using the PERT (also known as Beta PERT) distribution for each disease and syndrome. Variable distribution for each frequent disease or syndrome was assessed, and a Wilcoxon rank sum test was used to compare perceptions between producers and veterinarians.

Results

The largest percentage of producers and veterinarians were aged between 35 and 44 years (36.6%, 34/93), followed by those aged 25-34 years (26.9%, 25/93), 45-54 years (17.2%, 16/93), ≥55 years (15.1%, 14/93), and ≤24 years (4.3%, 4/93). In terms of gender and experience, the largest percentage of the participants were males (53.8%, 50/93) and had more than 20 years of experience (38.7%, 36/93), followed by 10-19 years (36.6%, 34/93), 5-9 years (17.2%, 16/93), and 1-4 years (7.5%, 7/93). For their perceptions, when considering all median values on the scale, the impact of frequent diseases or syndromes on calf health was highest for fracture (7/10), then, in decreasing order, septic arthritis (6/10), congenital defect (6/10), respiratory disease (6/10), diarrhea (5/10), dystocia (5/10), inadequate passive immunity transfer (5/10), the presence of a wound or abscess (4/10), and umbilical infection (4/10).

Veterinarians and producers had a similar perception for most of the selected indicators. However, veterinarians perceived a higher impact on calf health for dystocia, septic arthritis, and umbilical infection compared to producers ($P < 0.05$). The median for dystocia was 6.3 (interquartile range =IQR: 5.3, 6.7) for veterinarians and 4.7 (IQR: 3.7, 6) for producers. In the case of septic arthritis, the median was 6.7 (IQR: 6, 7.7) for veterinarians, while producers had a median of 4.8 (IQR: 3.7, 6). Regarding umbilical infection, veterinarians had a median of 4.7 (IQR: 4, 5.3), and producers had a median of 3.7 (IQR: 2.7, 4.8).

Conclusions

Producers' and veterinarians' perceptions of the impact of nine frequent diseases or syndromes on calf health were assessed. The impact of these diseases or syndromes on

calf health varies according to both the specific health problem and the perspectives of veterinarians and producers. In future steps, this measure can be combined with the number of disease or syndrome cases to determine the number of days lived with a disability. This information can guide strategic decisions on the farm based not only on the number of cases but also the impact on the overall health of the calves.

1246 - Days lived with disability due to diarrhea and respiratory diseases in dairy calves

Author: Jean Silva Ramos , Marianne Villettaz Robichaud, Jocelyn Dubuc, Débora Santschi, Jean-Philippe Roy, Gilles Fecteau, Sébastien Buczinski

Objectives

The life of a calf can be negatively affected by disease, leading to disability. Despite extensive exploration of this subject in human science, quantifying the number of days lived with a disability at the population level remains a challenge in animal science. On the dairy farm, access to this measure would be beneficial for developing cost-effective healthcare and welfare strategies.

This study aimed to quantify the number of days lived with disability (DLDs) due to diarrhea and respiratory diseases in calves during the preweaning period. We hypothesized that the number of DLDs due to diarrhea and respiratory disease in pre-weaned calves could be quantified by combining disease-related indicators such as the number of cases, duration, and the impact of these diseases on calf health.

Material and methods

This project received approval from the local ethics committees for the use of animals (Protocol number 21-Rech-2144) and for research in science and health (Protocol number 2023-4118). A total of 1815 pre-weaned calves from 40 dairy Québec farms were evaluated between February 2022 and August 2023. Diarrhea was assessed using a standardized clinical score ranging from 0 (solid) to 3 (liquid feces). Respiratory disease was evaluated combining parameters as rectal temperature, cough, ocular and nasal secretion, and ear carriage. All parameters ranged from 0 (normal) to 3 (severe alteration). Positive cases were defined for diarrhea (score 2 or 3) and respiratory disease (cut-off ≥ 5). All calves were evaluated by a veterinarian. The duration of diseases, accounting for the start time of treatment and the moment of recovery, was determined based on the literature. An average of 4 days was considered for diarrhea, while 7 days were considered for respiratory diseases. The impact of these diseases on calf health, measure as known as disability weight, was assessed in a previous study according to the perception of veterinarians and producers using a scale from 0 to 10. The obtained values were converted to a scale of 0 to 1. The estimated daily impacts of diseases were 0.5 for diarrhea and 0.6 for respiratory disease. An incidence-based methodology was used to obtain DLDs due to diarrhea and respiratory disease. This involved multiplying the number of positive cases by the average duration of a disease episode and the average impact of the disease on calf health. DLDs were described according to diseases, sex, and adjusted proportions based on 10 pre-weaned calves evaluated per farm.

Results

Diarrhea (n=507/1756, 28.9%) was the primary contributor to DLDs, followed by respiratory disease (188/1815, 10.4%). In general, 1804 DLDs were quantified for diarrhea and respiratory diseases in calves during the preweaning period. Stratifying by disease, 1014 DLDs were quantified for diarrhea, while a total of 790 DLDs were quantified for respiratory disease. Considering the sexes, 1534 DLDs were quantified for female calves, while male calves accounted for 270 DLDs.

Adjusting the DLDs for every 10 pre-weaned calves examined per farm, a median number of 8 DLDs was observed, with a minimum of 1 and a maximum of 22 DLDs. Looking at specific diseases, the median number of adjusted DLDs for diarrhea was 6 (min = 1, max = 9), and for respiratory disease, it was 3 (min = 0, max = 12).

Conclusions

The number of days lived with disability due to diarrhea and respiratory disease in dairy calves during the preweaning period was quantified. This health summary measure outperforms prevalence and incidence measures by considering not only the number of cases but also the duration and impact of diseases on animal health. As in human science, this measure can guide cost-effective strategic decisions for producers, veterinarians, and the industry, aiming to improve the health of the calves. In future stages, a greater number of diseases and days of life lost due to early death need to be considered to develop a global calf health summary measure.

1251 - The effect of individual quarter dry-off on milk production, occurrence of mastitis, and culling risk in Holstein dairy cows

Author: Haritha Somula , Ajay Singh, Madeleine Spellman, Matthias Wieland

Objectives
Individual quarter dry-off (QDO) has been increasingly employed as a strategy for managing cows with chronically elevated somatic cell count (SCC) and recurrent clinical mastitis. However, there is little knowledge about the influence of QDO on milk production, SCC, the risk of clinical mastitis, and the risk of removal from the herd. Therefore, this retrospective cohort study aimed to investigate these associations. Our working hypothesis was that QDO would decrease SCC without negatively impacting the milk production.
Material and methods
In this study, data from 471 dairy cows subjected to QDO were analyzed. The cows were housed on a 4,000-cow dairy farm in New York State with a thrice-daily milking schedule. The QDO was practiced based on fresh cow check, recurrent clinical mastitis, *Staphylococcus aureus* infection in a single quarter, and cows with chronic subclinical mastitis. Comparison cows (CON, n = 471) were matched with QDO cows in terms of parity and stage of lactation. Data were obtained regarding milk yield, SCC, clinical mastitis event after QDO, and the culling event. The SCC values were obtained for the 1 test day prior to (T-1), as well as 1, 2, and 3 (T1 to T3) test days after the event of QDO. Generalized linear mixed models with an identical link were used to estimate milk yield and SCC at T-1, T1, T2, and T3. We used proportional hazards regression models to study the hazard risks of mastitis occurrence and removal from the herd during the first 100 d after QDO. Statistical analyses were performed in SAS with the PROC MIXED and the PROC PHREG procedures. The cows were followed until 100 days in milk of the subsequent lactation.
Results
Following the QDO, the daily milk yield at the first milk recording at T1 was lower compared to the respective controls. Least squares means and 95% confidence intervals (95% CI) of milk yield at T-1, T1, T2, and T3, respectively, were 36.1 (35.0-37.3), 34.9 (34.0-35.9), 35.2 (34.3-36.1), and 33.6 (32.6-34.6) kg/d for cows subjected to QDO, and 41.0 (39.9-42.1), 42.8 (41.9-43.7), 41.7 (40.8-42.6), and 39.3 (38.4-40.2) kg/d for cows in group CON. For SCC (log_{10}-transformed), least squares means (95% CI) at T-1, T1, T2, and T3, respectively, were 5.4 (5.36-5.5), 5.1 (5.0-5.2), 5.1 (5.0-5.2), and 5.1 (5.0-5.2) for cows in group QDO, and 4.8 (4.7-4.9), 4.8 (4.7-4.84), 4.8 (4.7-4.9), and 4.8 (4.7-4.9) for cows in group CON. Proportional hazards regression models revealed associations between QDO and clinical mastitis occurrence and removal from the herd. Compared with cows of group CON, the risk of hazard (95% CI) of clinical mastitis occurrence was 1.92 (1.48-2.48) for QDO cows. The risk of hazard (95% CI) of culling was higher in cows subjected to QDO compared with cows of group CON [hazard ratio, 95% CI; 3.62 (2.65-4.93)]. About 70% of the QDO cows entering the subsequent lactation entered it with 4 lactating quarters that persisted for at least 100 days after calving, suggesting a fair recovery for QDO cows that were not culled.
Conclusions

Individual quarter dry-off resulted in a decrease in SCC without negatively impacting milk yield production. Cows subjected to QDO had higher risks of hazard of clinical mastitis occurrence and culling, compared with their herd mates. We conclude that QDO can be a viable strategy to manage cows with chronically elevated somatic cell count and recurrent clinical mastitis.

1258 - Benefits of vaccination against Streptococcus uberis on udder health performance on a Dutch dairy farm with on farm culturing

Author: Jessica Hartjes , Marc Tomàs Sánchez, Anne-Miek Timmermans

Objectives

Mastitis caused by *Streptococcus uberis (Strep. uberis)* has been diagnosed increasingly for the last years in Dutch dairy farms. This Gram-positive species is known to cause both clinical and subclinical infections, with some cases becoming chronic. The objective of this field trial is to analyze the efficacy of a monovalent vaccine against *Strep. uberis* (UBAC®, HIPRA) in the reduction of Clinical Mastitis (CM) at herd level in The Netherlands and its effect on bacterial pathogens cultured.

Material and methods

In August 2020, a Dutch dairy farm with 645 cows and a milk production of 11.000 kg milk/ cow/ year, started with on farm bacteriological culturing of CM cases to monitor the pathogens and implement selective antibiotic treatments. The bacteriological culturing was performed by trained farm personnel. In February 2021, the amount of CM cases caused by *Strep. uberis* increased. In January 2022, different management adjustments concerning the milking parlour and liners did not result in any decrease, moreover the *Strep. uberis* infections increased persistently. To control the situation, a whole herd vaccination with a monovalent vaccine against *Strep. uberis* (UBAC®, HIPRA) was implemented on May 10, June 7 and July 5 2022. This protocol was followed by a booster vaccination every six months. Since February 2023 concurrently the liners were changed every 2 months and more attention was paid to the sanitizing of the liners.

Farm personnel examined animals daily to detect CM cases based on clinical signs, including abnormal milk and/or a hard or swollen udder. All CM cases were documented and a pre-treatment aseptic milk sample from the affected quarter(s) was obtained for bacteriological culturing. Thereafter, animals received antibiotic therapy according to the corresponding farm-specific treatment protocols. A CM was only considered as a new case when it occurred more than 2 weeks after a previous infection of the same quarter.

Results

A strong decrease in CM cases is observed comparing the period before with the period after the three primo vaccinations. Moreover, we saw a 73% decrease in CM, changing on average from 30 to 8 cases per month, between January and July (2022 and 2023). The number of average monthly CM cases over the total period decreased significantly ($p=0.04$) from 22 per month before vaccination to 14 cases per month. The proportion of infected heifers decreased in the CM cases group by 31%, changing from 13% to 9% of the total CM cases.

The bacteriological culturing results show no increase in non-*Strep. uberis* pathogens and so no bacteriological shift on the farm occurred. It does show a strong decrease of 77% in *Strep. uberis* positive samples per month on average as of November 2022, 3 cases, compared to the period August 2021 to October 2022, 13 cases. Also, a strong decrease of 80% in non-*Strep. uberis* pathogens per month on average is seen as of March 2023, 3 cases, compared to the period August 2021 to February 2023, 15 cases.

The average bulk tank somatic cell count decreased by 35%, from 355.000 cells per ml to 229.000 cells per ml, one year after vaccination.

Conclusions

This field trial shows that vaccination of dairy cows with a monovalent vaccine against *Strep. uberis* (UBAC®, HIPRA) has a positive impact on udder health performance. The number of annual CM cases, average monthly CM cases and *Strep. uberis* positive milk samples decreased. Additionally, a lower somatic cell count of the bulk tank milk and a stronger decrease in heifers with a CM compared to the multiparous cows was observed. By keeping the heifers healthy with mastitis vaccination, it can contribute to a more sustainable way of farming.

1259 - Impact of vaccination against Mannheimia haemolytica leukotoxoïd and Histophilus somni on individual antibiotic treatments and slaughterhouse results on a Dutch rosé veal farm.

Author: Niels Groot Nibbelink, Jessica Hartjes , Wico Teeuw, Marina Solé Berga

Objectives

Since 2007 the veal calf industry in The Netherlands has invested a lot in reduction of antibiotic usage. The large differences in antibiotic use between veal farms, suggest that there are opportunities to increase the reduction of antibiotic use[1]. Reducing the impact of bacterial pneumonia and fibrinous pleuropneumonia (polyserositis) can be considered as one of the main drivers of antibiotic reduction in veal calves.

The aim of this comparative study was to evaluate the impact of vaccination with a vaccine against *Mannheimia haemolytica* leukotoxoïd and *Histophilus somni* (HIPRABOVIS® SOMNI/Lkt, HIPRA) on different production parameters such as antibiotic use, individual treatments and slaughterhouse results, in male Holstein Frisian veal calves..

Material and methods

On a Dutch veal calf farm with a total of 1300 calves, batches of approximately 440 calves each are reared and fattened. During the first 12 weeks after arrival, the calves are kept in a rearing unit and then transported to the fattening unit located on the same farm. Since this farm produces young rosé veal calves, the animals are slaughtered at 8 months of age.

Since season of receiving calves in the rearing unit is a risk factor for pneumonia and concurrent impacts the antibiotic usage, we compare batches reared in at the same time of the year[2].

Two batches of non-vaccinated 877 calves (Control) entering in June 2021 and February 2022 were compared with two batches of 876 vaccinated calves (HIPRABOVIS® SOMNI/Lkt, HIPRA) entering in June 2022 and February 2023. Three days after arrival the calves were vaccinated with an inactivated *Mannheimia haemolytica* leukotoxoïd and *Histophilus somni* vaccine (HIPRABOVIS® SOMNI/Lkt, HIPRA) and boostered three weeks later with a second dose. The primary variables monitored of these batches, were individual antibiotic treatments for pneumonia and slaughterhouse results.

Results

The number of calves diagnosed with pneumonia dropped form 39,68% to 22,12% significant by 44.25% in the vaccinated group ($p<0.05$). The number of calves treated individually with antibiotics for pneumonia dropped significantly by 52.54% in the vaccinated group and the number of calves treated multiple times with antibiotics for pneumonia (chronics, > 2 cases) decreased significant even by 57.84% in the vaccinated group.

As a result, total number of individual antibiotic treatments per animal for pneumonia decreased from 0.69 to 0.31 significant by 55.07% ($p<0.05$) in the vaccinated group. Recordings of treatment dates of the individual calves show also a clear difference of number of individual antibiotic treatments for pneumonia over time. More than 50% of the individual antibiotic treatments occurred the first 35 days on farm.

Regarding performance in carcass weight a significant difference was observed between calves in control group and calves in vaccinated group. Vaccinated calves had 3.96 kg

higher carcass weight at slaughter. Calves without individual antibiotic treatment are significant heavier at slaughter compared to calves with 1 treatment (-8.14 kg) and chronic cases (-17.19 kg) when 2 or more individual antibiotic treatments were needed ($p<0.01$).

Individual carcass deviations were registered after inspection in the veal slaughterhouse. The number of calves with visible pleuritis due to chronic pleuropneumonia decreased from 4.91% to 2.58% significant by 47.45% in the vaccinated group. The reduction in visible lung lesions by 29.79% was in the vaccinated group numerical, but not significant.

Conclusions

This comparative field study on a Dutch veal farm showed that vaccination in veal calves with a vaccine against *Mannheimia haemolytica* leukotoxoïd and *Histophilus somni* (HIPRABOVIS® SOMNI/Lkt, HIPRA) resulted in a significant reduction of morbidity and in number of individual antibiotic treatments for pneumonia. The number of calves with multiple individual antibiotic treatments for pneumonia was by 58% significantly less in the vaccinated group. As a result, vaccinated calves showed higher average carcass weights (+3,96 kg) and significant less calves showed lesions at slaughter related to pneumonia and fibrinous pleuropneumonia.

Also, the study proved that calves having an individual antibiotic treatment for pneumonia weigh significantly less at slaughter compared to calves without any history of individual antibiotic treatments for pneumonia.

[1]*Usage of antibiotics in agricultural livestock in the Netherlands in 2022, SDa/1156/2022, The Netherlands Veterinary Medicines Institute (SDa), 2023*

[2]*J. Bokma, R. Boone, P. Deprez and B. Pardon, J. Dairy Sci. 102: 607–618, 2019, Risk factors for antimicrobial use in veal calves.*

1266 - Successful treatment of idiopathic tetanus using metronidazole, magnesium, and acepromazine in Hanwoo (Korean indigenous cattle) yearling bull

Author: youngjun kim

Objectives

Bovine tetanus is a serious infectious disease of the central nervous system caused by the exotoxin produced by *Clostridium tetani* and is characterized by persistent tension and spasm of the rhabdomyocytes. Currently, many studies have focused on diagnosing tetanus; however, only a few studies on treatment methods have been conducted. Therefore, cattle with tetanus have been treated using symptomatic therapy. In this case, severe muscle spasticity and spasms were observed in a 9-month-old Hanwoo (Korean indigenous cattle) bull, and aspartate aminotransferase and creatine kinase levels were increased in serum biochemical tests. Clinically, bovine tetanus was strongly suspected, and metronidazole was administered orally for 5 days. To treat the intensifying bloat, a temporary rumenostomy was performed on the third day of onset, and the toxin gene (tetanospasmin) of *C. tetani* was amplified by polymerase chain reaction analysis from the collected ruminal fluid. Magnesium and sedatives (acepromazine) were administered for 7 days to treat muscle spasticity and spasms. Muscle spasticity and spasm markedly improved, and the bull stood up from the lateral recumbent position. On the 17th day after onset, all tetanus-related symptoms resolved and a normal diet was started. Our findings demonstrated that treatment with metronidazole, magnesium, and acepromazine was effective in the bull with tetanus.

Material and methods

A 9-month-old Hanwoo bull was found to have increased responsiveness to external stimuli, ears pulled back, and protrusion of the third eyelid (Figure 1A). At the time of onset, physical examination revealed the following: a body temperature of 38.9°C, heart rate of 92 beats per min, and respiratory rate of 61 cycles per min. The degree of spasticity of the hind limbs was insignificant, and no external wounds capable of inducing tetanus were observed. Although not confirmed, tetanus was strongly suspected, and 22,000 IU/kg of procaine penicillin (Procillin inj., Cheilbio, Korea) was intramuscularly injected; moreover, no sedatives were administered because muscle rigidity was not severe. On the second day, the spasticity of the neck muscles became severe, and spasticity of the hind limbs was noted. Mild ruminal bloat was observed with an elevation of the tail head (Figure 1B). Additionally, convulsions in the tail were conspicuously observed (Figure 1C). Therefore, typical tetanus was suspected based on these clinical symptoms.

Early onset changes in the clinical symptoms are summarized in Table 1. On the first day of outbreak on assuming a lateral recumbent position, the bull attempted to stand up on its own but failed. On the second day, the lateral recumbent position was maintained, and on the third day, the neck spasticity intensified and it was impossible for the bull to ingest feed on its own. The symptoms of muscle spasticity and convulsions gradually worsened; hence, the degree of tetanus was considered very severe.

On the third day of diagnosis, intensive care was started. The animal was moved to a dark place to minimize light and sound stimulation, and feed and water containers were hung at head height. Because the ruminal bloat was severe, an attempt was made to

expel gas through a stomach tube; however, this procedure failed due to increased tension in the laryngopharyngeal muscles. Therefore, a temporary rumenostomy was performed. The bull in the lateral recumbent position was erected using a cow lift. Then, a circular, 4-cm-diameter incision was made in the left waist, and a rumenostomy was performed using a cannula *via* a 50 mL syringe (Figure 2A). A cover was then installed on the rumen cannula for gas release and nutrient supply to relieve ruminal bloat and was maintained for 5 days (Figure 2B). Additionally, metronidazole (Metrojyl, JSK, Korea) was orally administered at a dose of 25 mg/kg every 12 h for 5 days to treat the infection.

Results

After the rumenostomy, the lateral recumbent position was reestablished, and 0.9% normal saline was infused according to the degree of dehydration (8%) to treat extensive azotemia, which was secondary to dehydration. Subsequently, 2 g of magnesium (Daihan Magnesium Injection 10%, Dai Han Pharm Co., Ltd., Korea) and 5 mg/100 kg of acepromazine (Sedaject, Samu Median Co., Ltd., Korea) were diluted in 500 mL of saline, and continuous intravenous infusion was administered for 20 min. Visually, the muscle spasticity decreased to some extent; however, no definite effect was observed. The concentration of serum magnesium before (0.9 mmol/L) and 30 min after (0.98 mmol/L) administration was not increased. After 6 h, 8 g of magnesium and 5 mg/100 kg of acepromazine were diluted in 500 mL of saline and re-infused intravenously for 20 min. Consequently, an increase in serum magnesium concentration (0.9 mmol/L before administration, 1.3 mmol/L in 30 min after administration) was observed, and the bull in the lateral recumbent position stood on its own with the improvement of muscle spasticity and convulsions. Additionally, the bull fed from the feeder at head height on its own.

Magnesium and sedatives (Magnesium sulfate 6 g + acepromazine 5 mg/100 kg) were administered at intervals of 6–8 h (3 times/day) for a total of 5 days and then at intervals of 12 h (2 times/day) for 2 days. For the first 2 days, only after the administration of magnesium and acepromazine, the bull could stand up from the lateral recumbent position and feed from the feeder hung at the height of the head. However, severe salivation and difficulty in chewing were still observed, and after 2–3 h, hind limb and tail convulsions recurred along with lateral recumbency. On the sixth day of diagnosis, the spasticity of the neck muscles gradually resolved, the pulled-back ear returned to its original state, and the third eyelid protrusion was restored. However, hind limb spasticity and spasm of the tail persisted. On the seventh day, the spasticity of the hind limbs was greatly alleviated, and no more ruminal bloats occurred; thus, the site where the rumenostomy was performed was closed. However, the patient was unable to eat the bottom feed and could only feed at the height of the head. On the 11th day, the spasm of the tail recovered; however, mild spasticity of the hind limb and elevation of the tail head were observed. On the 17th day, all the spasticity of the hindlimb and elevation of the tail head resolved completely, and the patient began to eat the bottom feed (Figure 5). The changes in clinical symptoms observed during tetanus treatment of the patient for 3 weeks are summarized in Table 3.

Conclusions

This study describes a recovered case of tetanus in a bull in the ROK. Our data demonstrated that metronidazole, magnesium, and sedatives (acepromazine) are effective in treating bulls with tetanus.

1280 - Field study to determine risk factors for milk leakage at drying off

Author: F. Leboeuf, J. M. Swinkels, M. Le Guenic, D. Fouvez, Tiago Tomazi

Objectives

Selective drying off protocols result in most cows being treated with an internal teat sealant (ITS) only, posing a risk for new intramammary infections (NIMI) during the dry period, if not managed properly. Although partial insertion of the ITS tube tip into the teat canal is recommended to prevent NIMI, 50% of French dairy farmers still fully insert the tube tip, as they perceive it prevents milk leakage (ML), also a well-known risk factor for NIMI.

The primary objective was to study the effect of full versus partial insertion of the ITS into the teat canal, on ML. The secondary objective was to identify and confirm other underlying, biologically plausible, risk factors for ML in the days after drying off.

Material and methods

Three French dairy farms, 2 with HF cows and 1 with Normande cows, were selected. At drying off, cows were treated at the cow level with either an ITS alone (Shutout® / Cepralock ®, MSD Animal Health) or combined with antibiotic according to on farm selective dry cow therapy protocol. The insertion technique of ITS was randomly performed at the cow level, with either the full or partial insertion of the tube tip into the teat canal.

Treatment was performed by trained technicians. ML was checked at the quarter level once, at 48 ±3h after drying off, and was defined at the quarter level by any visual milk presence at the teat end. At the same time, teat end callosity (TEC) was scored, using a 4-point scale score: normal (N), smooth (S), rough (R) or very rough (VR). Then, gentle pressure was put on the teat between 2 fingers, mimicking the natural pressure occurring during walking, or lying down, to see whether ML occurred. If ML was visual, it was also positive for pressure ML, but not the other way round.

Data were stored using online software (Get Feedback®), and univariable and multivariable statistical analysis was performed to identify associations with biologically plausible factors related to ML.

Results

Of the 202 cows included, 102 were inserted partially, and 100 were inserted fully with ITS. Average farm milk production at the last day before drying off was 11.2- 17.4 L /day, and the geometric mean at herd level of the last DHI SCC tests before drying off was between 48-178 cells/ml. On average, 11.6% of cows showed ML, 28.1% showed gentle pressure ML. Twelve % of quarters and 10.9% of cows showed a teat-end callosity score R or VR at drying off.

In the univariable model, both for cow level visual and gentle pressure ML, partial or full insertion was not a risk for ML (P = 1.0, and P = 0.7, respectively). Cow level TEC (R or VR), milk production at the first DHI test post-calving, and genetic index for milking speed and udder morphology, were significant contributors to ML ($P < 0.05$).

In the multivariable model, only TEC score R or VR versus N or S, in at least 1 teat at the cow level was a significant risk for gentle pressure ML (OR = 2.7, P = 0.007). Front quarters had a lower risk for ML compared to hind quarters (OR = 0.17, $P < 0.001$). Genetic index was not included in the final model because different breeds were used, but results were not consistent across farms.

Conclusions

Full insertion of the ITS tube tip into the teat canal did not pose a risk for ML, when compared to partial insertion and therefore, veterinarians should advice partial insertion. Instead, several udder health related genetic indexes and TEC were identified as a risk factor for ML. To mitigate the indirect risk of ML for NIMI in the short term, cows with TEC at drying off should considered to be additionally treated with antibiotic. In the long term, udder health related genetic indexes should be improved to decrease ML and to increase the efficacy of ITS when used alone.

1284 - Metabolic profile of Holstein × Gyr cows: effects of parity and body condition score at calving

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Objectives
This study aimed to evaluate the effects of parity and body condition score (BCS) at parturition on the metabolic profile of high producing Holstein × Gyr cows during the transition period.
Material and methods
This study was carried out in a dairy farm located in the state of Minas Gerais, Brazil. Ninety-six healthy dry Holstein × Gyr cows with approximately 240 days of gestation, 1/2 (77 cows) and 3/4 (19 cows) crosses, were included. The cows were kept in an open confinement system and grouped according to the production period: close-up cows (approximately 30 days before calving); fresh cows (from calving to 21 DIM); and lactating cows. Feeding consisted of total mixed ration, calculated for pre-calving and lactating cows (DCAD = −14.9 and 25.4 mEq/100g DM, respectively). The cows were distributed into three groups according to parity: primiparous (n = 20), biparous (n = 20), and multiparous (n = 20) with three to six calvings. All these cows had BCS 3.0−3.5 at calving. Another three groups were created according to the BCS at calving: high (HBCS; BCS > 3.5; n = 20), normal (NBCS; BCS = 3.0−3.5; n = 21), and low (LBCS; BCS < 3.0; n = 15). All cows in these groups were multiparous with three to six calvings. The NBCS group was composed of cows from the multiparous group, adding one more. Blood samples were collected and BCS was assessed on days -21, -7, 0, 7, 21 and 42 relative to parturition. Serum concentrations of NEFA, BHB, cholesterol, total protein (TP), albumin, BUN, Ca, P, and Mg were measured. Diseases during early lactation were computed as well as the occurrences of high lipomobilization, subclinical ketosis (SK), and subclinical hypocalcemia (SHC). Data were analyzed by two-way repeated measures ANOVA.
Results
Average daily milk yield varied from 24.6±4.2 to 28.4±5.0 kg/d. All metabolites studied varied over time (P < 0.001), except Mg concentration in parity groups (P = 0.062). Regardless of grouping BCS remained unchanged until parturition and decreased during early lactation; BHB, cholesterol, BUN, and TP increased during early lactation; NEFA concentrations were higher on the day of parturition and at 7 DIM; albumin and Mg fluctuated slightly; and Ca values were lower on the day of calving remaining low in early lactation. P values were lower on calving day in cows grouped according to BCS, but barely varied in cows grouped by parity. In cows grouped according to parity, differences between groups were observed for all metabolites, except P, however, it occurred in a few time points, being more frequent at 7 and 21 DIM. At 21 DIM, primiparous cows had lower BCS, BHB, cholesterol, and TP values, and intermediate values of NEFA and Mg. Compared to primiparous, multiparous cows exhibited lower values of Ca at calving. In cows grouped according to BCS at calving the BCS difference between groups was maintained at all time points. Except for minerals Ca, P, and Mg, differences between groups were observed for the other metabolites, mainly at parturition and during early lactation. HBCS cows had higher NEFA values than LBCS cows at calving, and higher BHB values than LBCS cows at calving and at 7 and 21 DIM. LBCS cows had lower

TP values than HBCS cows at -7 d and at calving, and lower albumin values than NBCS cows at calving. BUN concentrations differed between groups at parturition and in early lactation without a defined pattern. High lipomobilization was present in 6.2% (6/96) and 9.3% (9/96) of the cows in the prepartum period and in early lactation, respectively. Only 3 cows (3.1%) showed SK, and all were HBCS cows. At calving, 53.1% (51/96) of the cows had SCH. Among them 4 were primiparous, 7 biparous, and 40 multiparous. Twenty-four of these cows (25%) maintained SCH at 7 DIM. The diseases observed up to 42 DIM were metritis (21/96; 21.8%), clinical mastitis (17/96; 17.7%), and retained placenta (4/96; 4.1%).

Conclusions

It can be concluded that well-nourished high-producing Holstein × Gyr cows are, in general, metabolically balanced animals and that the parity and BCS at calving have no relevant effect on their metabolic profile. In multiparous cows, SCH on the day of calving can be highlighted as the most important imbalance.

1302 - Lameness and claw health of dairy cows in German compost bedded pack barns

Author: Phillip Andreas Guhl , Lisa Bachmann, Adrian Steiner, Maike Heppelmann

Objectives

A recent study describes lower prevalence for lameness and better claw health for cows housed in compost bedded pack (CBP) barns compared to freestall barns in Minnesota and eastern South Dakota (Lobeck et al., 2011). Therefore, the aim of this study was to investigate the influence of CBP barns on lameness scores and claw health in German dairy herds.

Material and methods

Eight farms with CBP barns in south Germany were visited from January to August 2023. All farms had managed their dairy cows for a minimum of two years in CBP barns without pasture access and have regular claw trimming visits. Data were collected during the claw trimming visit of the herd. All cows were scored for lameness on a scale from 1 to 5 where cows with a score from 3 to 5 are considered lame. Claw findings were diagnosed based on the “ICAR Claw Health Atlas” using the program “KLAUE” by dsp agrosoft (Egger-Danner et al., 2020). All claw diagnoses were digitalised in Microsoft Excel to calculate a claw health score for every cow and farm based on geometric severity scores (Kofler et al., 2023).

Results

The prevalence of lameness (median±mean absolute deviation [MAD]; lameness score >2) detected in this study for all 612 scored cows was 8.9 ± 4.0%, with 7.8 ± 2.9% scoring a 3 and 0 ± 0% scoring a 4. A score of 5 was not detected. In summary 91.1 ± 4.0% of the cows were not lame, with 77.7 ± 4.4% scored a lameness score of 1 and 13.3 ± 2.7% scored a 2. The calculated median claw health score for every cow (n=640) was 8 ± 8 and for the farms 9 ± 3. The prevalence (n=640 cows) of sole ulcers was 3.4 ± 3.5% (mean±SD), of white line abscesses 0 ± 0 % (median±MAD) and of digital dermatitis 10.7 ± 12.1% (mean±SD).

Conclusions

Cows of this study showed a lower lameness prevalence in comparison to cows housed in freestall cubical barns as described for southern Germany (23.6%) (PraeRi, 2020). The prevalence for claw lesions such as sole ulcers, white line abscesses and digital dermatitis are lower in CBP barns, than in studies with freestall barns (Jury et al., 2021). To summarize, CBP barns promote high percentage of lameness-free cows and favorable claw health.

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1321 - Impact of vaccination against Bovine Viral Diarrhea Virus at the beginning of fixed-time AI programs on the reproductive performance of beef cattle in Argentina

Author: Pedro Bermudez, Mauricio Lopez di Lena, Emanuel Gumina, Demian Bellido, Andrés Wigdorovitz

Objectives

The objective of the present study was to evaluate an alternative vaccination protocol on fixed-timed artificial insemination programs in Argentinian beef cattle.

Material and methods

Three experiments were performed to evaluate the effect of vaccination against bovine viral diarrhea virus (BVDV) on the reproductive performance of beef cows without (experiments 1, 299 heifers) or with previous vaccination against this disease (experiments 2 and 3, 183 and 236 cows respectively). Cows were assigned to a fixed-time AI protocol (FTAI; d −11 to 0) in all experiments. Pregnancy status was determined with transrectal ultrasonography on day 35 and 90 after AI. Pregnancy loss was considered in cows pregnant on d 35 but non-pregnant on the subsequent evaluation.
Animals were randomly divided in two groups, vaccinated group (VAX) received two doses of a subunit targeted vaccine against BVDV on days -11 and 90 while Control group (CON) remained did not receive the vaccine. Three different experiments were carried out on commercial farms in the province of Salta, in the northern region of the country. In all the experiments, the same personnel and semen from the same bull were used:
Experiment 1, 149 Brangus heifers were in VAX group and 150 in CON group.
Experiment 2, 102 Brangus cows were in VAX group and 82 in CON group.
Experiment 3, 146 Brangus cows were in VAX group and 90 in CON group.

Results

In experiment 1, pregnancy rates were increased at day 35 (VAX= 55%, CON 48%, P=0,20) and pregnancy loses were significantly reduced (P = 0.0014) in VAC (5%) heifers compared with CON (22%).
In experiment 2, pregnancy rates were increased at day 35 (VAX=53%, CON=42%, P=0,18) and pregnancy loses were significantly reduced (P = 0.0001) in VAX (9%) cows compared with CON (15%).
In experiment 3, pregnancy rates were increased at day 35 (VAX=54%, CON=41%, P=0,06) and pregnancy loses were reduced (P = 0.59) in VAX (3%) cows compared with CON (5%).
Taken together the results of the three experiments, pregnancy rate was significantly increased (P=0.0087) and pregnancy loses were significantly reduced (P=0,0007). As a result, at day 90 post FTAI 51,5% (205/398) of VAX animals and 37,2% (119/320) of CON animals were pregnant (P=0,0002).

Conclusions

The standard recommendation in vaccination programs is to vaccinate animals 60 to 45 days prior to IA protocols or breeding season. However, this recommendation has some practical limitations in Argentinian livestock production, particularly in nursing cows and in farms with substantial land extensions, where the movement of animals within the farms is difficult. Applying vaccines at the beginning of the FTAI protocols, the same day the intravaginal device (IVD) is inserted, could be a practical solution, since less animal

movement is required. Previous investigations in Brazil have shown promising results in this direction but there was no information in Argentine cattle (Ferreira et al., 2018; Lima et al., 2010; Pereira et al., 2013). These results show two things: first, that vaccination and initiation of AITF programs can occur simultaneously, saving movement and confinement of animals which is a crucial issue in nursing cows and in some locations of our country; and second, they show, once again, the importance of controlling BVDV infections since they affect the reproductive performance (pregnancy rates and in pregnancy losses) of heifers and cows.

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1324 - Improving herd health together

Author: Ann-Kristin Nyman, Paulina Lingers

Objectives

In order to increase health of our production animals, preventive actions are needed as neither antimicrobials nor vaccines removes the factors causing disease. Due to all research performed a lot of knowledge how to prevent disease exists, but it is not always so available or easy to use for farmers. And it is farmers, or their employees, that must execute the actions on farms to prevent disease. To be able to present knowledge in a way that is easy to grasp and transform it into actions has always been a great challenge for advisors, either it is a veterinarian or feed advisor or any other person that are advising. With the aim to make advice more accessible and easier to work with, the development of a tool started in Sweden 15 years ago by Växa (the largest dairy farm advisory service company in Sweden (farmer owned)).

Material and methods

We had the idea to organize and summarize knowledge by breaking down areas of importance for different health issues into several building blocks that together takes most knowledge about how to prevent that specific issue into account. You work with each block separately, and the lay-out of the entire area is a pyramid. You start at the bottom to make sure that the foundation is stable, and then deal with each building block in the right order so you don't miss factors that are of importance to accomplish before moving to the building blocks above. In some farms the foundation is already there, and they can start working higher up in the pyramid, while others must start with the foundation. Everyone can use this way of working to improve animal health in their herd.

Each building block contains an evaluation of one or more key indicators that can be useful to detect areas in that specific building block that needs attention. These are followed with a list of things to do to succeed with a particular block. In the end of each block, you can find extra tips on things you could do more of in each area to make improvements. The information given in the pyramids are based on national and international research as well as practical experience from dairy farms in Sweden. The thresholds of the key indicators are mainly based on national statistics from the Swedish milk and disease recording schemes.

Results

Through a lot of hard work, good co-operation, and development, we have now a full function web tool containing 10 pyramids. You can work with areas like hoof health (claw horn lesions and infectious claw lesions), injuries and lesions, feeding, transition period, fertility, and udder health (contagious and environmental infections in conventional and VMS systems, respectively). The advice, key-indicators and thresholds given in the pyramids might not be suitable for all counties/regions/farms to use. However, as the areas mentioned in the pyramids are of importance for the health issues considered they can still be used to start the preventive work and be a way for farmers and advisors to join and start a discussion about a subject and then combine the working method with regional recommendations and thresholds.

Conclusions

The developed web tool is easy to use for both farmers and advisors. The stepwise layout encourages to work with a smaller area at a time and helps (reminds you?) not to miss important areas on the way upward. There are no explanations included in this tool on why you should perform a certain measure, and this is because the discussion between the farmer and the advisor is an important complement to this tool. To work preventive will increase animal health and production as well as reducing the use of antimicrobials, increasing the probability of cows with a longer lifespan for a sustainable future.

1335 - Summer scour syndrome – an emerging disease of dairy calves at pasture: case definition and histopathological lesions

Author: Maresa Sheehan, John Mee, Seamus Fagan, Ciara Hayes, Donal Murphy, Donal Lynch, Eoin Ryan, Diana Bochynska, Maire McElroy, Shane McGettrick

Objectives

Summer scour syndrome (SSS) is an emerging disease of dairy calves in their first season at pasture. It has been documented in Ireland, the UK, New Zealand and in Australia. It is likely it is also present in other pasture-based dairy countries. The objective of this presentation is to highlight its occurrence in order to encourage networking with interested colleagues internationally. Here we present a consensual case definition and a review of the histopathology of affected cases.

Material and methods

A series of web-based expert consultations was conducted in 2023 to find a consensus on the case definition internationally for this syndrome. Colleagues from Ireland, the UK, New Zealand and Australia participated. In addition, twenty-five cases of suspected SSS were identified in the government (DAFM) veterinary diagnostic laboratories database based on the clinical history, age, gross findings, and lack of aetiological agents. Samples were collected from various organs at full necropsies and processed for histopathology. The cases were reviewed and scored based on the histopathological changes by 5 independent pathologists. This was preceded by discussions with stakeholders from Teagasc (**Teagasc** is the state agency that provides research, advisory and education in agriculture, horticulture, food and rural development in Ireland),University College Dublin (UCD), Veterinary Ireland (**Veterinary Ireland** is a representative body for **veterinary** surgeons in Ireland), Volac (Dairy Nutrition) and the Regional Veterinary Laboratories (RVLs) as well as experts from the UK, New Zealand, and Australia to incorporate their experience/knowledge of the anamnesis, gross pathology and histopathology.

Results

Case Definition: SSS may be defined clinically by diarrhoea and upper alimentary tract ulceration in at least some animals in a group of weaned dairy calves less than 12 months of age on a grass-based diet. This is a diagnosis of exclusion. To exclude other common causes of these clinical signs a complete anamnesis along with individual faecal and blood samples should be collected from 10-12 affected animals in the affected group to exclude coccidiosis, parasitic gastroenteritis, infectious agents (BVD, MCF, fungi, others) and copper- molybdenum imbalances. Grass sampling for mineral and trace element analysis is also recommended.

Histopathological findings: Mucosal lesions were seen in the buccal mucosa/oesophagus and rumen. Commonly identified lesions include apoptosis/dyskeratosis, cellular and intercellular oedema, colour patterning affecting all layers of the epithelium. Furthermore, some cases exhibited a degree of basal cell necrosis.

Conclusions

A consensual clinical case definition, with recommended sampling, will improve diagnostic agreement and reliability. The histopathological profiling of Irish SSS cases will allow trans-national comparisons of cases to confirm/refute their similarities and hence possible common aetiologies and preventive measures.

1342 - SURGICAL TECHNIQUE FOR RESOLUTION TYPE III PERFORATED ABOMASAL ULCERS

Author: João Silva, Paulo Capêlo, André Martins, Ana Claúdia Coelho, Miguel Quaresma

Objectives

Describe the technique used during abomasal displacement surgery to identify and correct type III abomasal ulcer. Type III abomasal ulcers are perforated ulcers that may cause localized peritonitis. Surgery is recommended for these lesions, but the prognosis depends on the location and size of the lesion and the type of peritonitis present (Niehaus, 2016).

Material and methods

It is part of the daily clinical activity of a veterinarian who works with dairy cattle to perform surgeries to correct abomasum displacement. Still, of the surgeries performed, three abomasum displacement surgeries stood out, in which type III perforated abomasal ulcers were found. Two of these abomasum displacements were on the left, which made it more complex to reposition the abomasum in the correct place without spilling contents into the abdominal cavity; the third case was an abomasum volvulus.

In all three cases, the perforated ulcer was located in the abomasum's greater curvature, allowing for better exteriorization and possible surgical intervention. In addition to this aspect, another factor in making exteriorization easier was the size of the incision, which should be as large as possible in order to have the ulcer on the outside. A tobacco pouch suture was first used to correct the abomasal ulcer to prevent liquids from leaving the abomasum. Next, where the abomasal mucosa was still visible, isolated horizontal mattress stitches were made to invaginate and promote better healing of the site. Finally, an Utrecht suture was used to properly isolate the ulcer site and minimize the risk of peritonitis. This suture was used for two purposes: to reduce the outflow of exudate and liquids from the ulcer site and to minimize the exposure of the stitches and exogenous material as much as possible in order to reduce the risk of peritonitis. For these sutures, ¾ catgut thread and 3/8 circle 68 mm round section needles were used.

Once the ulcer wad resolved, the abomasum was fixed using pyloro-omentopexy with 3 USP Catgut wires. Before closing the incision, the abdominal cavity was washed with Ringer's lactate (4 to 6 L), and Benzylpenicillin procaine, and Dihydrostreptomycin. The muscle layers were sutured separately with a simple continuous suture using a catgut and a 3/8 round needle. The skin was closed with ford interlocking suture, a Supramid, and a triangular section needle 3/8 of a circle.

After surgery, a combination of Benzylpenicillin procaine and Dihydrostreptomycin (1.5 mL per 25kg p.v.) was administered intramuscularly for five days, metamizole sodium monohydrate (40 mg/kg, intravenously) for five days and meloxicam (0.5 mg /kg, once intravenously). In the two cows with left-sided abomasal displacement, only antibiotic therapy and pain control were given in the days following surgery, and they didn't present any other type of post-surgical complications.

In the case of the cow with the abomasal volvulus, blood transfusion (1L) and intravenous fluid therapy (4L) were carried out 6 hours after surgery due to the state of shock and blood loss during surgery. The case was reassessed 24 hours after surgery, and it was decided to administer intravenous fluid therapy with Ringer's lactate,

Sterovet®, and Soroplasma®, and oral fluid therapy via the orogastric tube. Just 48 hours after the surgery, the cow began to drink and eat. This animal was euthanized two weeks later due to trauma to the right hind limb, and a necropsy was carried out, which showed good healing after surgery and no peritonitis.

Results

Of the 3 cases presented, all were clinical successes, despite the difficulties and adversities in carrying out this type of surgical intervention in the field and all the complications that can arise in the post-surgical period.

Conclusions

Despite the success of these cases, the prognosis may be poor in other similar cases. The post-surgical process should include adequate pain control and fluid therapy support, both intravenous and oral, so that the animals start eating as soon as possible after surgery to achieve the best possible recovery.

1346 - The potential of the tail tip as animal health indicator in dairy cows

Author: Amalie A. Köhler, Lea M. Lorenz, Armin M. Scholz, Christine Schmidt, Mirjam Lechner, Prisca V. Kremer-Rücker

Objectives

Tail tip inflammation and necrosis is a well-recognised condition in several species that can have a significant impact on overall health. Fattening cattle, bulls and calves have often been prone to severe tail tip problems, often related to their housing conditions or environmental factors. In addition, a high frequency of tail tip lesions has recently been observed in dairy cattle. Tail tip changes have also been studied in pigs in relation to the newly defined Swine Inflammation and Necrosis Syndrome (SINS). Pathological changes including inflammation and necrosis of ears, teats, claws and tails have been observed in pigs as a result of systemic inflammation and reduced blood flow to the acra. This raised the question of whether changes in the tip of the tail were also associated with other health indicators in dairy cows. The aim of this study was therefore to investigate whether there is a correlation between changes in the tail tip, its temperature measured by thermography, and performance and health traits in Holstein Friesian dairy cows.

Material and methods

The study was conducted on a commercial dairy farm in Germany between May 2021 and June 2022. Every 14 days groups of 19-43 cows were examined. A total of n=530 observations from 351 different Holstein cows (lactation = 1 - 6) were analysed during the study period. Cows were examined at 150 days post partum and/or just before drying off. Data collection included examination of the tail tip for tail tip lesions (TT), annular lesions (AN), hyperkeratosis (HK), swelling (SW) and axis deviation (AD); pain sensation at the tail tips, body condition score (BCS) of the cow, the locomotion score (LMS) and the somatic cell count obtained from a current California Mastitis Test (CMT). Rectal temperature (BT) was measured and cows were clinically examined with particular attention to ruminal filling (Rum_{fill}) and intensity of contractions (Rum_{int}). In addition, a thermographic image of each tail tip was taken to record the maximum ($Temp_{Max}$), minimum ($Temp_{Min}$) and average temperature ($Temp_{Ave}$) of the tail tip. The claws were trimmed and the diagnoses were converted to a claw score (Sum_{Claw}). Data from the milk performance testing as milk solids (MS), protein % (MP), fat % (MF) and somatic cell score (SCS) were also included in the statistical analysis. Cluster analysis using SAS version 9.4 was performed to identify possible patterns among the variables and resulted in 4 clusters.

Results

The highest coefficient of determination, i.e. the greatest effect on clustering, was Sum_{Claw} (R^2=0.68), followed by $Temp_{Ave}$ (R^2=0.63), $Temp_{Min}$ (R^2=0.60), $Temp_{Max}$ (R^2=0.55) and CMT values by quarter (R^2=0.26-0.47). The first cluster stood out with the worst values for CMT and SCS. MP and MF were high due to low MS. Meanwhile, AN, HK and BCS were highest. Rumen values were above average, as were tail tip temperature and the LMS, but Sum_{Claw} was low. The cluster with the highest Sum_{Claw} also had the worst LMS, the lowest values for Rum_{fill} and Rum_{int}, and the highest values for TT, SW, AD and PS. Tail temperatures were well above average. Milk yield was low and udder health was the second worst of all groups. The cluster with the highest MS, and therefore lowest

MF and MP, also showed lowest CMT and SCS. Tail alterations were below average, except for HK. In this group, tail tip temperatures were highest and so were Rum_{fill} and Rum_{int}. For the last cluster, the coldest tail tip temperatures were outstanding in contrast to the highest BT. TT, SW, AD were the second highest, while AN and HK were the lowest. In addition, Sum_{claw}, LMS and Rum_{fill} and Rum_{int} had low values.

Conclusions

An important finding was that Sum_{Claw} and tail tip temperatures strongly influenced cluster categorisation, both of which could have a significant impact on animal health. Furthermore, in cases of udder and claw issues, the tail tips presented the most severe alterations and the lowest temperatures. The tail tips of cows with good performance and minimal health issues remained warm and unaltered. Therefore, the results of this study indicate a correlation between the tail tip and different animal health traits that requires further investigation.

Funding

Parts of this research were funded by the Tönnies Forschung, Rheda, Germany.

1348 - Relationship between blood gas analysis and Somatic Cell Count in Holstein-Friesian cows

Author: Chiara Tommasoni, Enrico Fiore, Anastasia Lisuzzo, Giorgia Taio, Antonio Barberio, Eliana Schiavon, Matteo Gianesella

Objectives

Mastitis represents one of the main pathologies in dairy cattle farms. Diagnosis can be performed based on somatic cell count equal to or higher than 100.000 cells/mL, presence of clinical signs and positivity to microbial cultures. Portable blood gas analyzer represents already for many diseases and species a reliable and fast tool in order to asses timely the clinical status of the animals, but it's role and utility in case of dairy cows' mastitis is still controversial. According to this, the objective of this study was to assess the role and utility of blood gas analysis in case of mastitis.

Material and methods

This study was carried out within the Agritech National Research Center. For this study, 61 Holstein Friesian dairy cows have been enrolled from a single dairy farm in the Veneto Region-Italy having high incidence of mastitis, in order to avoid bias due to environment or management conditions. Animals with antibiotic treatment prior the evaluation, or presenting other diseases differing from mastitis were excluded. Animal care and procedures were in accordance with the European directive 2010/63/EU and the national law D.L. 2014/26. During this study, sterile pool of 4-quarter milk samples were examined for microbial analysis and SCC, and clinical examination of all quarters and animals was performed. After 6 hours from milking, blood gas analysis was performed. 1 mL of venous blood was collected from the jugular vein and analyzed with a portable blood gas analyzer. Statistical analysis was performed using the SAS system. Normal distribution of data was evaluated. For non-normally distributed data, Kruskal-Wallis test was performed. One Way Anova was performed for normally distributed data, instead. Mean values and relative standard error (SE) were calculated between groups for each parameter. P-value<0,05 was considered significant. For the first statistical analysis, animals were divided, based on SCC into 2 groups: "low" (34 animals), having SCC< 100.000 cells/mL, and "high" (27 animals), having SCC ≥100.000 cells/mL. Only lactate showed a non-normal distribution. Secondary, animals were divided based on positive or negative microbial culture into two groups: "positive" (52 animals) and "negative (9 animals). Anion Gap, hemoglobin, glucose, bicarbonate, hematocrit, lactate and sodium were not normally distributed.

Results

From microbiological culture, 9 animals resulted negative, 33 animals were positive to coagulase-negative staphylococci, 9 to *Streptococcus uberis*, 4 to *Enterococcus* spp., 2 to *Escherichia coli*, 1 to *Bacillus* spp., 2 to coagulase-positive staphylococci, 1 to *Klebsiella*. From the first statistical analysis, glucose and creatinine showed a significant difference between low and high groups. Mean value and relative SE was calculated. Glucose (expressed in mg/dL) for low group showed a mean value and relative SE of 4,83±0,47; high group showed 4,83±0,47. Creatinine (expressed in mg/dL) for low group showed a mean value and relative SE of 0,81±0,03; high group showed 0,69±0,02. Considering the statistical analysis between negative and positive microbial culture, chloride and strong ion difference (SID) showed significant differences. Chloride (expressed in mmol/L)

showed respectively for negative and positive group mean values and SE of 98,7±1,23 and 101,4±0,58; SID, instead, showed values of 46,7±0,86 and 40,2±0,58.

Conclusions

From the statistical analysis, glucose showed a significant decrease in the group with high levels of SCC. Previous studies highlighted similar results. Possible explanations might be first of all the limited supply of blood sugar due to the direct influx of blood flow to the infected udder; secondary, glucose might be transported from the milk to the extracellular pathway to maintain the osmotic balance between the extracellular environment and the milk with the increase of sodium and chloride. Creatine was also significantly decreased in the "high" group. After being synthetized, creatinine is released to the blood serum and cleared by the kidneys, representing, in association with blood urea, an important index of renal functionality. However, it might also be influenced by hydration status and muscle activity. Considering that only creatinine was significantly altered, this might be related to external factor such as animal behavior before the sampling. Results obtained with the second analysis might be due to the highly inhomogeneous consistence between positive and negative groups. Further studies need to be performed.

1352 - Impact of assisted calving and retained fetal membranes upon milk production in farms under a smallholder production system

Author: Luis Javier Montiel-Olguín, Héctor Raymundo Vera-Ávila, Mario Alfredo Espinosa-Martínez, Eliab Estrada-Cortés, Felipe de Jesús Ruiz-López

Objectives
The objective was to determine the effect of assisted calving and Retained Fetal Membranes (RFM) on milk production during early lactation and 305-d Milk Yield (305MY) in farms under a small-scale production system.
Material and methods
The study was conducted in the Los Altos region, located in the state of Jalisco, in western Mexico (20°49'01"N, 102°43'59"W, 1800 meters above sea level). The farms included in the study met the characteristics typical of smallholder dairy production system in Mexico, as reported in previous studies. A total of 23 farms were included, and 99% of the cows included in the study were of the Holstein breed. Milking was carried out twice a day, and monthly milk weighing was conducted over a period of 18 months. The following variables were analyzed: assisted calving (yes/no), presentation of RFM (yes/no), number of lactations (1, 2, and 3+), and sex of the calf (male/female). The response variables analyzed were milk production at 30 days in milk and 305MY. All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). For the final analysis, 279 lactations with complete information for the variables of interest were used. Mean comparisons and contrast tests were performed using the PDIFF option in the GLM procedure. The model included main effects and second-order interactions. The level of statistical significance was set at P≤0.05, and for statistical trends, P≤0.1 was considered significant.
Results
For milk production during early lactation the number of lactations was significant (P≤0.05), while the RFM factor tended to be significant (P≤0.1). No other main effects or interactions were found to be significant or showed statistical trends. Cows in their first, second, and third or more lactations produced 16.9±1.4, 21.6±1.8, and 22.1±1.6 kg/day, respectively. Cows without RFM produced 21.8±0.8 kg/day, while cows with RFM produced 18.6±1.7 kg/day. For 305MY, assisted calving had a significant effect (P≤0.05). Cows without assisted calving had higher 305-d production compared to cows that required assistance (6,587.5±211 kg and 5,518.9±491 kg, respectively). The interactions assisted calving x number of lactations and RFM x sex of the calf approached significance (P≤0.1). There was no difference (P>0.05) in 305MY due to the number of lactations in cows without assisted calving (6,472±419 kg, 6,590±399 kg, and 6,700±237 kg for cows with 1, 2, and 3+ lactations, respectively). However, in cows with assisted calving, cows in their first and third or more lactations had different 305MY (P≤0.05), with cows in their second lactation having average values (6,204±577 kg, 5,927±851 kg, and 4,426±820 kg for cows with 1, 2, and 3+ lactations, respectively). Furthermore, the 305MY of cows without RFM that gave birth to a female calf (6,899±288 kg) was higher (P≤0.05) than those of cows that had RFM (5,385±599 kg) or cows that gave birth to a male calf without or with RFM (6,032±301 kg and 5,896±592 kg, respectively).
Conclusions

In conclusion, RFM impacts milk production during early lactation (~-3.2 kg/day) and calving assistance negatively impacts 305MY (~-1,069 kg/lactation), with a greater effect observed in cows in their third or subsequent lactations. Acknowledgments: SIGI Project 15352034772.

1357 - DAIRY FARMER'S PERCEPTION ABOUT BOVINE RESPIRATORY DISEASE (BRD) ON THEIR FARMS AND ITS REAL IMPACT

Author: João Silva, Alfredo Teixeira, Paulo Capêlo, André Martins, Miguel Quaresma, Ana Cláudia Coelho

Objectives

The main purpose of this study was to improve producers' understanding of infectious diseases, such as identifying Bovine Respiratory Disease (BRD) in the calves on their farms and analyzing the owners' perception of the seriousness of the problem. The idea was also to provide suggestions for improving management and protocols for vaccination or treatment to achieve better results than those previously observed.

Material and methods

For this study, we used a convenience sample of 5 farms in the municipality of Barcelos, Portugal, with a previous history of BRD in calves under 100 days of age. A visit was made to these farms to identify the problems, and a lung ultrasound was also done on all calves under 100 days of age. Lung ultrasound is a minimally invasive diagnostic method that allows the identification of BRD more sensitively, in contrast to auscultation, which has some limitations in the diagnosis of lung consolidation (Buczinski et al., 2014). The echographic assessment was recorded according to the TUS Score.

In addition to the lung ultrasound, an epidemiological survey was done to understand the most critical moments for BRD and the owner's perception of the impact of BRD on their farm.

After the survey, thoracic ultrasound, and assessment of risk factors, an improvement plan was drawn up for each farm to reduce BRD and reduce its impact on the calves and, consequently, on the future of the farm.

Results

From the survey of producers' perceptions, 3/5 thought they had more problems with BRD in the first 21 days of calves' lives, 1/5 between 44 and 65 days, and 2/5 between 22 and 43 days since on one farm they thought they had a BRD problem in 2 periods of the survey (0-43 days). About the type of prevention done during rearing to prevent BRD, 3/5 only did intranasal vaccination, 1/5 did intranasal and parenteral vaccination, and 1/5 did not do any type of prevention. The time of year when there was more BRD was 2/5 in the winter, 1/5 only in the summer, 1/5 in the spring, and 1/5 in the fall. As for the presence of BRD on the farm, 3/5 considered that they only treated up to 25% of the animals for BRD, while 2/5 treated between 76 and 100%.

Sixty-eight ultrasound assessments were carried out, averaging 14 per farm. Of these 68, 29% of the calves (20/68) were considered to have severe lesions, 32% (22/68) had mild lesions, and 38% (26/68) had no lesions. The average number of animals without lesions in the five farms evaluated was 45.6%. One farm, in particular, had 64% (7/11) calves with severe lesions.

Conclusions

The results of this study suggest that BRD occurs on the dairy farms in the study. According to the survey, 3 of the five farms mentioned that they only treated less than 25% of the animals, but in the ultrasound evaluation, 3 of the five farms had more than 25% of the animals with lung lesions. Producers only treat part of the animals affected

by BRD since it is more prevalent on farms than the treatments carried out. It is also often present in subclinical situations not identified in time.

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1372 - Calf management practices in Polish dairy herds based on farmers' survey

Author: Korytkowski Łukasz, Wojciech Młocek, John Mee, Paweł Górka, Jadwiga Flaga, Mohammed Kabiru Baba, Zygmunt M. Kowalski

Objectives
Since there are no published reports on the procedures used in calf rearing in Poland, the aim of our study was to survey the procedures of calf management from birth to weaning. We focused on farms located in Central-Eastern Poland, where the calf rearing is mostly performed by the farmers themselves or members of their family.
Material and methods
Data were collected on farm characteristics and calf management practices from a self-selecting sample of 453 dairy farmers (owners or employed staff). The farms were located in 5 provinces of Central-Eastern Poland. The data were collected at seminars on calf rearing held for farmers from October 2017 to December 2020. The questionnaires were administrated to farmers before the oral presentations. The questionnaires were completed voluntarily by respondents with the help of co-authors of this abstract. The questionnaire consisted of closed or opened questions divided in 5 sections, i.e. management and housing of calves, colostrum management, colostrum feeding, milk or milk replacer feeding and health of calves. Based on the number of lactating cows in the herd at the time of the survey, the farms were retrospectively divided into three herd size groups: small (<20 cows; 122 herds), medium (21 to 50 cows; 213) and large (≥51 cows; 118). The answers for each question were analyzed using herd size as the grouping variable. All computations were conducted using R (R Core Team, 2022) within the RStudio development environment. Testing was performed at a significance level of α=0.05. Because empirical cell frequencies in the contingency table were not always at least 10, independence was tested by the Fisher's exact test. P-values were computed through Monte Carlo simulation with 105 replications.
Results
Herd size was not associated with the time after calving that calves are separated from their dam, testing of calf serum protein concentration (yes/no), feeding the calves with waste milk (yes/no), feeding the same milk replacer throughout the liquid feeding phase (yes/no) and criteria used for weaning (P>0.05). However, other variables were associated with herd size and for most of them, the smaller the farm, the worse the calf-rearing practices. On 24% of farms, the calves were kept outside (cold rearing; more so in larger farms, P<0.001), mostly in individual pens or hutches (55.7%; P<0.001), and some (14%) on a chain. On 89.4% of farms first colostrum was milked out immediately after calving or calf drying. On the majority (82.6%) of farms, colostrum quality was not assessed, although this was more likely to occur on larger farms (P<0.001). However, in almost 90% of farms, colostrum was administered early, i.e. within 2 hours after birth. Administration of first colostrum by an esophageal tube was not common (3.7%), but more likely to occur on larger farms (P<0.001). On the majority of farms, calves were fed using buckets with/without (42.8/55.4%) a teat and only on 1.8% of farms were calves fed in automatic feeding stations. The most common daily volumes of milk or milk replacer were 4-6 L (39.4%) and 6-8 L/d (43.9%). On most farms (82%) hay or other forages were offered in preweaning period, but diets without hay were more likely to

be offered in large farms (P<0.001). Offering texturized starter feed alone or with whole grain was more common on large farms (P<0.001). In total 34% and 27% of calves were weaned at 5-6 or 7-8 weeks of age, respectively, with no effect of farm size. Regardless of the farm size, the most important criteria for weaning of calves were their age and body weight. Only in 5% and 11% of farms, respectively, were passive immunity and body weights assessed. The main health problems in the surveyed farms were diarrhea (69.5%), followed by pneumonia (23.3%). Diarrhea was also the main reason (42.6%) for calf deaths.

Conclusions

This study showed that many of the practices used in rearing dairy calves on the surveyed farms were sub-optimal. Moreover, for most variables studied, the smaller the farm, the worse the calf-rearing practices. These findings have implications for targeting of education programs, in particular, prioritization of farmers with smaller dairy herds.

1405 - Abomasal Ulcers in Dairy Cattle: Brazilian scenario

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Objectives

Abomasal ulcer is a disease that can be present and go unnoticed or can lead to clinical manifestations that, despite being non-specific, can evolve and lead to the death of the animal. In ruminants, the diagnosis of this disease has been challenging, since the technique considered the gold standard, endoscopy, remains viable only for monogastric animals. Although some Brazilian studies report the presence of ulcers in cattle, these are limited to descriptions of clinical cases or necropsy findings. Thus, the objective of this study was to investigate the occurrence of ulcers in dairy cows culled from slaughterhouses, focusing on the quantification, classification, and location of lesions.

Material and methods

The study involved the analysis of the abomasums of 120 dairy cows of different ages, from a dairy region located in the northwest of the state of Sao Paulo/Brazil. After the desensitization, bleeding and evisceration process, the abomasums were isolated and carefully opened along the greater curvature. Macroscopic analysis of the mucosa was conducted to identify the presence, number, and location of ulcers (Munch *et al,* 2019). Ulcers were classified according Braun et al (1991). Samples of the mucosa and ulcers were collected for subsequent histological examination, to confirm the type of ulcer found.

Results

Of the 120 abomasums analyzed, 108 (90%) had ulcers, totaling 2,013 lesions. Among these ulcers, 310 (15.40%) were type 1A; 1,401 (69.61%) type 1B; 169 (8.40%) type 1C; 43 (2.14%) type 1D; 31(1.54%) type 2; and 59 (2.93%) type 3. Regarding location, the highest number of ulcers was found in Zone 1, totaling 1,144 ulcers. In this location, 863 (75.43%) type 1B lesions were observed; 131 (11.45%) type 1A ulcers; 65 (5.68%) type 1C; 36 (3.15%) type 1D; eight (0.70%) type 2; and 41 (3.58%) type 3. In zone 2, 254 lesions were observed, of which 12 (4.72%) were type 1A ulcers; 212 (83.46%) type 1B; five (1.97%) type 1C; four (1.57%) of type 1D; 19 (7.48%) type 2; and two (0.79%) of type 3. In zone 3, 609 ulcers were observed, of which 326 records (53.56%) were type 1B; 166 (27.27%) were type 1A; 99 (16.25%) type 1C; , 11 (1.81%) type 3; 4 (0.66%) were type 2; and 3 (0.49%) were type 1D. In addition to ulcers, other conditions in the abomasum were observed such as edema, abomasitis, reddish, whitish and purplish aspects/points in different points of the abomasum and in the proximal duodenum.

Conclusions

This study identifies the occurrence of abomasal ulcers in cull dairy cows, highlighting the predominance of type 1B lesions, associated with hemorrhage in the abomasal mucosa. Zone 1, comprising the body and fundus abomasal region, shows the most affected region. This analysis emphasizes the importance of these ulcers in dairy cows, demonstrating their potential impact on health, well-being and animal production. These results reinforce the need for early and in vivo diagnosis strategies to reduce the effects of these ulcers on the health and production of ruminants.

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1411 - Effects of sprays with and without antibiotics on wound healing after disbudding of calves

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Objectives
Disbudding of calves is standard practice in the dairy industry as horned adult cattle will injure each other and are dangerous for farmers and other handlers on the farm. Disbudded horns are often treated with wound sprays, to aid healing and prevent or treat infections as infections will affect the progression of the healing process. Many sprays contain antibiotics, but there is also a range of wound sprays without antibiotics. To reduce antibiotic use, wound sprays with antibiotics should only be used when there is no equivalent alternative available. To this end, we performed a clinical study to assess whether an wound spray without antibiotics (Keno™fix) was superior or equivalent to CTC-spray.
Material and methods
Between October and November 2019, 255 calves on 37 farms were included in the study. After disbudding, the two wounds were semi-randomly treated with either (Keno™fix) (NWS) or CTC-spray (AWS). We used the diameter of the wounds and a lesion score as primary outcome variables and measured these respectively with a caliper and an existing scoring system at week 1 and 4 after disbudding.
Results
The mean healing speed from week 0 – 4 was 1.6 (±0.1) mm/week. NWS treated wounds healed slower than AWS treated wounds, with 1.6 (± 0.1) mm/week vs. 1.7 (± 0.1) mm/week. Wounds treated with NWS had a higher risk for a poorer wound score when compared to CTC treated wounds at both 1 week (30% vs. 10%, respectively) and 4 weeks (22% vs. 12%, respectively) after disbudding. For both treatments, a higher wound score at week 4 was associated with a slower wound healing speed in mm / week from 0 – 4 weeks.
Conclusions
We concluded that the small difference in the speed of wound healing between NWS and AWS is clinically not sufficient to support routine treatment of disbudding wounds with CTC-spray. The difference in mean healing speed between NWS and AWS is likely explained by the higher prevalence of infected wounds. We confirmed that this is associated with slower wound healing.

1421 - Occurence of abomasal ulcer in Brazilian pasture beef cattle

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Objectives

Abomasal ulcers are an important disease that leads to significant economic losses such as decreased productivity and animal death. Despite the importance of the disease a gap regarding Brazilian beef cattle still exists. Therefore, the present study aims to determine the status of abomasal ulcers in brazilian grazing beef cattle. Classify lesions type, number, and topographic distribution. Describe the occurrence of the disease considering sex (entire male/ castrated male/ female), age (1;2;3;4 ≥ 5 years), lesions type (1A; 1B; 1C; 1D; 2; 3; 4; 5) and topographic distribution (Zone 1/Zone 2/Zone 3).

Material and methods

An observational crossover study has been conducted in a commercial slaughterhouse located in Campo Grande – MS. After slaughter, the abomasum was separated from the rest of the gastrointestinal tract and identified. Followed by the opening through the greater curvature, removal of the contents, washing and macroscopic evaluation of the abomasal mucosa. All visible ulcers and abomasal lesions were counted, located considering the division of Munch et al. (2019) and classified according to Whitlock (1980), Smith, Munson and Erb (1983) and Braun, Eicher and Ehrensperger (1991). Breeding system, sex and age information was provided by the slaughterhouse. A descriptive analysis has been conducted.

Results

A total of 191 abomasum have been evaluated. Of the samples 79.1% (151/191) were female and 20.9% (40/191) were male; 82.5% (33/40) were entire and 17.5% (7/40) were castrated male. Regarding age, 7.9% (15/191) were up to one year old, 26.2% (50/191) two years old, 22.5% (43/191) three years old, 6.3% (12/191) four years old, 37.1% (71/191) five years old. 86.4% (165/191) had lesions in abomasum while 13.6% (26/191) did not have. Considering topographic region 36.5% (60/165) were only in zone 1, 3.0% (5/165) only in zone 2; 10.3% (17/165) only in zone 3; 4.8% (8/165) in zone 1 and 2; 30.9% (51/165) in zone 1 and 3; 2.4% (4/165) in zone 2 and 3; and 12.1% (20/165) in all zones. In respect to the classification of ulcers 139 animals had ulcer type 1A, 112 had type 1B, 2 had type 1C, 8 had type 1D and 2 had type 3. Were counted 3,256 lesions of which 46.3% (1508/3256) were ulcer type 1A, 52.7% (1715/3256) were type1B, 0.1% (4/3256) were type 1C, 0.8% (27/3256) were type 1D and 0.1% (2/3256) were type 3. No type 2, 4 and 5 were found.

Conclusions

The present study showed that abomasal ulcers are present in the Brazilian beef cattle grazing herd. Type 1A is the most common lesion however animals with type 1B have greater number of lesions. Future studies will show the real impact of type 1A and 1B ulcers on the health and performance of animals.

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1422 - Case report: Use of Blitz therapy with 3rd and 4th generation cephalosporins as a method of eradicating Steptococcus Agalactiae on a dairy farm in Brazil.

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Objectives

Streptococcus Agalactiae is recognized as one of the main causative agents of mastitis in dairy cattle in the world. It is known that the transmission of *S. Agalactiae* occurs mainly from cow to cow during the milking process through direct contact between infected animals, contaminated milking equipment, or by the hands of the milker. Mastitis caused by this kind of bacteria is highly contagious, but also demonstrates an excellent response to treatment with intramammary antibiotics, and therefore, this condition becomes a strong candidate for the implementation of a rapid and mass treatment strategy to eradicate this type of bacteria. Blitz-therapy is a treatment that consists of the use of intramammary antibiotics in all quarters of lactating cows and has become a popular approach in herds with high somatic cell counts due to the significant prevalence of *S. Agalactiae*, mainly as a form to control the spread of this bacteria. This work reports a successful case of using Blitz-therapy to treat and eliminate subclinical mastitis caused by *S. Agalactiae* on a dairy farm in the north of Rio Grande do Sul, state with great dairy importance for Brazil. The property had a somatic cell count (SCC) above 783,000/mL in June 2023, along with a high prevalence of positive bacterial cultures for the mentioned bacteria.

Material and methods

Given the high frequency of new cases and chronic cases quickly diagnosed through individual culture plates, associated with a tank CCS higher than desired for milk quality, it was decided to work with Blitz-therapy in this dairy herd. This approach aims to stop the spread of the pathogen by carrying out mass treatment in all animals with a positive culture for the bacteria, regardless of the presence of symptoms or CCS value. The process began with bacterial culture on all 114 cows in the lactation batch, using individual milk samples, which were collected by the veterinary team that serves the property and sent for analysis to a veterinary laboratory in Botucatu (SP). In the first test, 54 cows were diagnosed as positive for *S. agalactiae*, with 13 of them excluded from therapy because they had been dried or sent to slaughter before laboratory results. In the first treatment, a ceftiofur intramammary antibiotic was administered for 3 consecutive days in the 41 positive cows that remained in the lactation group and in another 24 that gave birth after collection, totaling 65 animals treated. After 10 days, a second analysis, now with 129 lactating animals for testing, revealed that only 6 animals remained with a positive result, and 14 new cows were infected, so a treatment with cefquinome intramammary antibiotics was then carried out in these 20 animals. After another 10 days, a new analysis of the herd, now with 126 animals in the lactation group, showed only 1 positive diagnosis, resulting in its discard due to the chronic nature of the mastitis.

Results

After 3 months of treatments, the SCC in the property's tank was 484,000/mL in the dairy control, decreasing to 256,000/mL after 5 months, a value considered close to ideal for the average tank of a herd with good udder health.

Conclusions

It was concluded that Blitz-therapy, with only 2 treatments of 3rd and 4th generation cephalosporins intramammarily, promoted bacteriological cure in the analyzed herd, evidenced by the absence of bacterial growth suggestive of *S. agalactiae* in subsequent sample cultures which had more than 200,000 CCS/mL in monthly milk production controls. The success observed in therapy in this case report strengthens its recommendation for the treatment of this type of mastitis, in addition to acting as a shielding measure for healthy animals in the herd.

1447 - Association of Serum Calcium Levels in the First Week Postpartum and Early Culling of Dairy Cows

Author: Luis Felipe Ruiz García, Rocío Silvia Sandoval Monzón

Objectives
To determine the association between serum calcium levels in the first week postpartum and the early culling of dairy cows.
Material and methods
The study was conducted in three dairy farms in the province of Lima over a period of 10 months. A sample of 398 Holstein dairy cows was used. During the study, all calving cows were monitored. Intensive production conditions were maintained, including feeding cows with a total mixed ration based on forage and concentrate. The study design was prospective longitudinal, following cows from calving to 100 days in lactation. The methodology included measuring serum calcium levels using blood samples. Calcium levels were assessed using a spectrophotometer and a commercial diagnostic kit. The determination of the early culling rate was made by reviewing records of cows culled during the first 100 days in lactation. Statistical analysis included the use of IBM SPSS Statistics 25, with ROC curve analysis to determine the optimal threshold for serum calcium values and a Cox proportional hazards regression model to assess the relationship between serum calcium levels and early culling, considering variables such as age at calving, season, and parity. Results were considered significant at a level of significance less than 5%.
Results
The animals included in the study had an average milk production at 305 days of 8597.35 ± 1979.85 kg, with a peak lactation milk production of 35.05 ± 6.35 kg/day, an average age of 3.64 ± 1.73 years, and an average of 2.43 ± 1.51 calvings per cow. During the transition period, cows require more minerals, especially calcium, to support lactogenesis in early lactation (Goff, 2014). The percentage of cows with early culling in the first 100 days postpartum was 7.50%. Various causes of premature death in cows were identified, with 31.58% due to emergencies, 28.95% to sudden death cases, followed by peripartum-related causes (21.05%), locomotor problems (15.79%), and others (2.63%). Regarding the determination of the serum calcium threshold for the risk of early culling, ROC curve analysis was performed, establishing an optimal threshold of ≥ 6.5 mg/dL for early culling, with a sensitivity of 34% and specificity of 84%. Additionally, it was found that cows with calcium levels below 6.5 mg/dL have a significantly higher relative risk of early culling (Hazard ratio = 2.48). The survival curve showed that multiparous cows have a higher risk of early culling in the first 100 days postpartum. Time-dependent relative risk assessment indicated that cows with serum calcium levels below 6.5 mg/dL in the first week postpartum have a significantly higher risk of being culled for health reasons during the first 100 days. This finding supports the importance of monitoring calcium levels in the first week postpartum to prevent the early culling of cows. Other authors also found a higher risk of culling during the first days of lactation when serum calcium concentrations were lower (Seifi et al., 2011; Roberts et al., 2012).
Conclusions
Conclusions:

There is a relationship between serum calcium levels and the early culling of dairy cows, establishing an optimal threshold of ≥ 6.5 mg/dL. Survival analysis indicated that cows with calcium levels below 6.5 mg/dL in the first week postpartum had a significantly higher relative risk of early culling, with a Hazard ratio of 2.48.

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1449 - Kinetics of two copper salts (copper-calcium and copper-zinc edetate) injected parenterally

Author: Esteban Galarza , Julián E. Jaeschke, Santiago J. Raggio, Maximiliano Itterman, Juan C. Muriel, Gabriel Rojas, Luis E. Fazzio, Guillermo A. Mattioli

Objectives

Parenteral supplementation with copper (Cu) salts is a common and effective practice for the prevention and treatment of Cu deficiency in cattle. These salts differ in their rate of transfer from the injection site to the liver, which is directly proportional to the risk of Cu toxicity (liver necrosis). In this sense, salts with a lower transfer rate are safer, but tend to cause reactions at the injection site. At present, Cu edetates are the salts of choice for current products. Although edetates usually do not cause local reactions, they have a high transfer rate and are usually indicated at doses of 0.3 mg/kg body weight, considering that 0.9 mg/kg already causes liver necrosis. Copper edetates are bound to other minerals such as calcium (Ca) or zinc (Zn) to form Cu-Ca and Cu-Zn edetates, respectively. Although both edetates are considered to exhibit similar transfer rates, they have not been compared in kinetic studies. The objective of the present experiment was to evaluate the plasma kinetics in calves of two commercial products with different salt-associated minerals (Cu-Ca edetate and Cu-Zn edetate).

Material and methods

Sixteen pre-weaned Aberdeen Angus calves (14 females and 2 males), 4 months old and 175.6 ± 19 kg live weight were used. The calves were randomly assigned to one of two groups. Group T1 (n=8, 7 females and 1 male) received a dose of a concentrated suspension of Cu-Ca edetate (25 mg Cu/mL; 1 mL/83.33 kg body weight) equivalent to 0. 3 mg Cu/kg body weight (new product in the Argentine market) and group T2 (n=8, 7 females and 1 male) received a dose of a Cu and Zn edetate solution (15 mg Cu/mL; 1 mL/50 kg body weight) equivalent to 0.3 mg Cu/kg body weight (Suplenut®, Biogénesis Bagó, Argentina). Both products were administered subcutaneously in the lateral region of the neck. Blood was collected by jugular venipuncture into heparinized tubes at time 0 (before supplementation) and at 20, 40, 60, 90, 120, 180, 240, and 360 minutes after parenteral supplementation. The samples were stored refrigerated and centrifuged to obtain plasma, which was stored at -20°C until its determination by flame atomic absorption spectrophotometry at the Mineral Nutrition Laboratory (FCV-UNLP). The design was completely randomized, and statistical analysis was performed with a linear regression mixed model with repeated measures (MIXED, SAS 9.4). The fixed effects were treatment ("group"), time ("minutes"), and the interaction of time x treatment. Animal was used as a random variable. When the time x treatment interaction effect was observed, the SLICE option was used to determine differences. Values are expressed as least squares means (LSMEANS) ± standard error of the mean (SEM), obtained using the LSMEANS option of the statistical program. A p-value ≤ 0.05 was considered significant for the main effect and for the interaction.

Results

At the beginning of the experiment, both groups showed plasma Cu values considered below the reference values (T1: 13.6±5.7 and T2: 14±4.6 µg/dL; RV > 60 µg/dL). The maximum value observed, for both groups, was 20 minutes post-application, when group T1 presented a higher concentration of Cu than group T2 (98±5.7 vs. 66.9±4.6

µg/dL, respectively; $p<0.01$), while in the rest of the times sampled the concentration of Cu in plasma was decreasing progressively and without differences between the groups ($p>0.05$). There were no local reactions in any of the treatments. Although both salts maintained average plasma Cu levels within the physiological range (60-120 µg/dL), the difference observed 20 minutes after parenteral supplementation suggests that they behave differently.

Conclusions

The higher plasma Cu level at T1 would indicate that Cu-Ca edetate would transfer Cu to the liver more rapidly than Cu-Zn edetate, increasing the risk of liver toxicity. Although the toxic dose of 0.9 mg/kg body weight has been established for Cu-Zn edetate, further experiments are needed to determine the dose at which toxicity is expected to be observed for Cu-Ca edetate.

1450 - Impact of Subclinical Hypocalcemia on Milk Production in the First Third of Lactation in Intensively Managed Cows in Lima, Peru

Author: Luis Felipe Ruiz García, Rocío Silvia Sandoval Monzón

Objectives
To determine the impact of subclinical hypocalcemia on accumulated milk production, peak milk production, and time to peak milk production during the first third of lactation in cows.
Material and methods
The study was conducted in three intensive dairy farms in the southern province of Lima, Peru, housing a total of 1500 production cows. A detailed follow-up was performed on at least 500 cows from calving to 100 days of lactation, excluding those with a history of clinical hypocalcemia or other conditions. Blood samples were taken in the first postpartum week to assess serum calcium levels, and milk production data were collected from monthly milking controls and individual animal records. Serum calcium levels were evaluated using a spectrophotometer and a commercial diagnostic kit. Normocalcemia (>8 mg/dL of serum calcium), mild subclinical hypocalcemia (<8 – >6.8 mg/dL of serum calcium), and severe subclinical hypocalcemia (< 6.8 mg/dL of serum calcium) were identified. The analysis focused on the impact of calcium levels on accumulated milk production, peak lactation production, and time to peak production in the three farms. A linear model was used to evaluate these variables. Statistical analysis included the comparison of the mentioned variables between cows with and without subclinical hypocalcemia using the t-Student test with a significance level of 5%.
Results
The results reveal the significant impact of severe subclinical hypocalcemia on milk production at 100 DIM under different conditions and categories of cows during the first third of lactation. Cows with severe subclinical hypocalcemia obtained significantly lower accumulated milk production than normocalcemic cows (2998.6 ± 139.7 kg vs. 3228.7 ± 119.0 kg), with no difference in peak lactation production or time to reach peak production. There was also no statistically significant difference with cows experiencing mild subclinical hypocalcemia. Additionally, primiparous cows achieved significantly lower accumulated milk production than multiparous cows (2637.2 ± 121.1 kg; 3296.4 ± 128.2 kg; and 3454.4 ± 114.8 kg, respectively) and peak lactation production (32.1 ± 1.3 kg/day; 37.8 ± 1.3 kg/day; and 40.6 ± 1.2 kg/day, respectively). Regarding calving season, cows calving in summer had lower accumulated milk production (3229.9 ± 73.0 kg vs. 3028.8 ± 144.3 kg) and peak lactation (37.8 ± 0.8 kg/day vs. 35.9 ± 1.4 kg/day) than cows calving in winter. Therefore, primiparous cows calving in summer and experiencing severe subclinical hypocalcemia (2548.1 kg at 100 DIM and 30.8 kg/day at peak lactation) showed lower accumulated milk production and peak lactation production compared to multiparous cows calving in winter with normocalcemia (3598.0 kg at 100 DIM and 42.1 kg/day at peak lactation). These results underscore the importance of addressing subclinical hypocalcemia to optimize milk production in cows during the critical lactation period (Rodríguez and Bach, 2017). Another study supports this result, finding that subclinical hypocalcemia impacts milk production and susceptibility to other diseases, leading to direct and indirect economic losses for producers (Reinhardt et al., 2011).

Conclusions

Severe subclinical hypocalcemia significantly decreases accumulated milk production at 100 DIM but does not affect peak lactation production or the time to reach peak production.

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1457 - EVALUATION OF TRANSFER OF PASSIVE IMMUNITY IN DIFFERENT SIZED DAIRY FARMS IN TURKEY

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Objectives

In the prevention of calf diseases, transfer of passive immunity (TPI) is very important. The incidence of morbidity and mortality increases in calves with failure of transfer of passive immunity (FTPI) and failure of transfer of passive immunity range from 10-40% worldwide. The most important factors affecting this are colostrum quality and the amount of colostrum given. The purpose of this study was to evaluate TPI in different sizes of dairy farms.

Material and methods

In the study, the blood serum samples of a total of 725 calves from 65 farms from 11 different provinces and regions of Turkey were used. The serum samples were collected from Holstein, Simmental, Jersey and crossbred calves at 1-5 days of age. The blood samples were grouped into 7 groups according to dairy farms size (Group I (≤20), Group II (21-100), Group III (101-200), Group IV (201-300), Group V (301-500), Group VI (501-1000) and Group VII (>1000)). The number of serum samples obtained from the groups I, II, III, IV, V, VI and VII were 30, 46, 39, 99, 108, 180, 223, respectively. The rates of primiparous and multiparous are similar in each group. The TPI was evaluated in blood sera by digital Brix refractometer (Milwaukee MA882, Hungary) and Brix % < 8.5% was considered to be a FTPI. The statistical evaluation of the FPTI rates between the groups was carried out using the Pearson chi-square test and the Fisher exact test.

Results

While the FTPI rate for the total of 725 calves was 20.3%, the FTPI rates for Groups I - VII were 26.7%, 41.3%, 30.8%, 11.1%, 20.4%, 26.1%, and 12.6%, respectively. According to these results, the FPTI rate was higher in group I (<20) compared to group IV (p 0.044). The FPTI ratio was found to be higher in Group II (21-100) compared to Group IV (p<0.001), Group V (p 0.007), Group VI (p 0.043) and Group VII (p<0.001) with higher animal numbers. In Group III (100-200), FTPI rate was higher than in Group IV (p 0.005) and Group VII (p 0.004). Group VI (501-1000) had a higher FTPI rate than Group IV (p 0.003) and Group VII (p 0.001).

Conclusions

According to the results, the highest FTPI rate was found in Group II (21-100) at 41.3%. In general, farms with fewer cows had higher rates of FTPI. This may be due to inadequate colostrum management skills of farm owners or workers in small farms. The fact that the FTPI rate was higher only in group VII compared to large farms can be explained by the higher number of FTPI found in Simmental calves in one farm sampled in this group. As a result, it can be seen that the rate of FPTI is lower in large farms with veterinarians and technical support. Colostrum management on small farms needs to be improved.

1464 - Calves with diarrhea up to 75 days of age have lower performance until weaning

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Objectives
When raising calves, diarrhea is a significant health issue. It affects their overall performance and leads to higher mortality rates and treatment costs. In this study, we aimed to assess the incidence of diarrhea and its impact on the performance of 303 calves from primiparous Nelore (*Bos indicus*).
Material and methods
The calves were born on a commercial farm in Bataguassu, MS, Brazil, during the 2022/2023 calving season. All animals were weighed at birth (D0), 30 days (D30), and 60 days (D60), as well as at weaning (D210). Body measurements were taken of each calf, including wither height, rump diameter, and thoracic circumference at D0, D30, and D60. Moreover, any occurrence of diarrhea was documented during the first 75 days of the calves' lives. Therefore, the calves were classified into three groups: Group A, animals that showed no clinical signs (n=57); Group B, animals with one or two diarrhea episodes (n=137); and finally, Group C, animals with three or more diarrhea episodes (n=109). All calves with diarrhea were treated with antibiotics, costing $1.48 per treatment. Group A received no treatment, while Group B and Group C received 1.49 and 3.98 treatments per calf, respectively. The following variables were analyzed: weight at four different periods (at moments D0, D30, D60, and D210) and wither height, rump diameter, and thoracic circumference at three different periods (at moments D0, D30, and D60). The data was analyzed using PROC GLIMMIX from SAS through repeated measures over time.
Results
There was an interaction group*time for weight (P=0.0017). Calves in group A presented greater weight when compared to calves in group C on D30 (73.1±2.43 vs. 65.2±1.79 kg) and D210 (170.7±2.96 vs. 158.9± 2.14 kg). However, no difference was observed between the groups on D0 (31.4±0.50 vs. 30.7±0.36 kg) and on D60 (110.6±2.89 vs. 93,2±2.15 kg). A group effect was observed for the wither height (P=0.0264), regardless of time. Calves in group A had greater wither height than calves in group C (D0= 70.1±0.60 vs 68.7±0.34 cm; D30= 83.1±1.11 vs 81.9±0.60 cm; D60=90.6±1.14 vs. 89.2±0.71 cm, respectively from group A and C),. The rump diameter had an interaction group*time (P=0.0014). On day 0, the diameter of the calves in group A was larger than the calves in group C (15.0±0.14 vs. 14.6±0.10 cm). However, on day 30, the measurements of calves from groups A and B were larger than those of calves from group C (22.2±0.47, 20.9±0.30, and 19.7±0.39 cm, respectively). On D60, calves presented the following measurements: 23.3±0.51, 23.3±0.43, and 23.4±0.36 cm for groups A, B, and C, respectively. Finally, thoracic circumference showed a group*time interaction (P=0.0566). On D30, calves in group A had greater thoracic circumference than the calves in group C (101.0±1.28 vs. 96.4±0.93 cm). However, there were no differences for D0 (72.5±0.51 vs. 71.8±0.38 cm) and D60 (110.6±1.15 vs. 108.2±0.89 cm). The costs of treatments were US$0.0, US$2.20, and US$5.89 for groups A, B, and C,

respectively. The mortality rate of the calves was 2.6%, and there was no significant difference between the groups.

Conclusions

In conclusion, calves that experienced three or more episodes of diarrhea up to 75 days old had lower weaning weights (11.8 kg) and higher treatment costs (US$5.89) than those that did not have diarrhea.

1489 - Characterizing the Accuracy of SASUB™: A 3-hour On-farm PCR Test for Detecting the Presence of DNA from Staph aureus or Strep uberis in Milk

Author: Roger Saltman , Jane Pascar, Jackie Corbett

Objectives

SASUB is a reagent manufactured by [Acumen Detection Inc., Syracuse, NY] that is used to determine the presence or absence in bovine milk of oligonucleotides from *Staphylococcus aureus* and/or *Streptococcus uberis* when used in the [Acu-Polaris] 3-hour on-farm and laboratory PCR system. This study used a robust number of samples of various concentrations of *Staph aureus and S. uberis* spiked into known pathogen-free milk (based on complete extraction lab PCR) to define the Limit of Detection (LOD), Limit of Quantification (LOQ), and Sensitivity of the test.

Material and methods

Inocula of live *Staphylococcus aureus* (ATTC 12600) were prepared:

1. Positive Sample 1 = 1 x 10^4 CFU/mL in milk (40 vials).
2. Positive Sample 2 = 1 x 10^3 CFU/mL in milk (40 vials).
3. Positive Sample 3 = 1 x 10^2 CFU/mL in milk (40 vials).
4. Negative (Control) Sample = Known negative (pathogen-free) milk (40 vials).

Additionally, inocula of live *Streptococcus uberis* (ATTC 700407) were prepared:

5. Positive Sample 1 = 1 x 10^4 CFU/mL in milk (40 vials).
6. Positive Sample 2 = 1 x 10^3 CFU/mL in milk (40 vials).
7. Positive Sample 3 = 1 x 10^2 CFU/mL in milk (40 vials)
8. Negative (Control) Sample = Known negative (pathogen-free) milk (40 vials)..

Sample Numbers for all inoculated tubes were randomly assigned using a random number generator in Excel. Tubes were labeled with the random Tube Number, Species of Inoculum, and Concentration of the Samples and this information was logged in a Master Sample Key file. All “Sample Tubes” were refrigerated and 287 tubes were randomly selected and transferred to a second Lab Technician who was blinded as to sample type and concentration.

Results

Criteria used to determine results:

True Positive (TP) = PCR result is POSITIVE for known Positive Inocula.
True Negative (TN) = PCR result is NEGATIVE for known Negative Inocula.
False Positive (FP) = PCR result is POSITIVE for known Negative Inocula.
False Negative (FN)= PCR result is NEGATIVE for known Positive Inocula.
Sensitivity = TP/ (TP + FN)
Specificity = TN/ (TN + FP)
Positive Predictive Value = TP/(TP + FP)
Negative Predictive Value = TN/ (TN + FN)

<u>Channel 1 (*Staph aureus* 1 x 10^2 CFU/mL) Results</u>

True Positive (TP) = 104
False Positive (FP) = 0
True Negative (TN) = 180
False Negative (FN) = 3

Sensitivity = 97%
Specificity = 100%
Positive Predictive Value = 100%
Negative Predictive Value = 98%
Because of the very high values for Sensitivity and Specificity at the lowest dilution of *Staph aureus* (1 x 10^2 CFU/mL) tested, the **Limit of Quantification (LOQ) for SASUB for Channel 1 was determined to be 1 x 10^2 CFU/mL** The **Limit of Detection (LOD) was at some dilution of Staph aureus lower than that.**

Channel 2 (*Strep uberis* 1 x 10^3CFU/mL) Results

True Positive (TP) = 34
False Positive (FP) = 1
True Negative (TN) = 177
False Negative (FN) = 1
Sensitivity = 97%
Specificity = 99%
Positive Predictive Value = 97%
Negative Predictive Value (Specificity) = 99%
Because of the high value for Sensitivity and high value for Specificity at the middle dilution of *Strep uberis* (1 x 10^3 CFU/mL) tested, the **Limit of Quantification (LOQ) for SASUB for Channel 2 was determined to be 1 x 10^3 CFU/mL** The **Limit of Detection (LOD) was determined to be 1 x 10^2 CFU/mL.**

Conclusions

The use of the SASUB reagent in a 3-hour on-farm PCR system for detecting oligonucleotides associated with *Staph aureus* and *Strep uberis* yielded excellent results. For *Staph aureus,* the LOD was below the dilutions tested and the LOQ was 1 x 10^2 CFU/mL. At the LOQ, the Sensitivity was 97% and the Specificity was 100%. For *Strep uberis,,* the LOD was 1 x 10^2 CFU/mL and the LOQ was 1 x 10^3 CFU/mL. At the LOQ, the Sensitivity was 97% and the Specificity was 99%. These results indicate that using the SASUB reagent with a 3-hour on-farm PCR may be a useful tool when evaluating the presence of *Staph aureus* or *Strep uberis* oligonucleotides in milk samples.

References

Saltman, R. et al. " Comparing the Accuracy of Two Diagnostic Tests, a 3-hour On-farm PCR System and Microbiological Culture, for Detecting the Presence of Mycoplasma bovis in Milk. " Abstract at 2023 NMC Annual Meeting, Atlanta, GA.

1490 - Calving & calf care management workshop – 3. Colostrum management: Setting the calf and producer up for success

Author: Dave Renaud , John Mee, Otto Szenci

Objectives

The objective of this workshop is the academic interchange of knowledge on topics pertaining to the periparturient/neonatal period affecting the dam and her offspring with emphasis on knowledge exchange, not lecturing. This abstract, one of three, focusses on colostrum management.

Material and methods

The workshop format will be that of the World Café methodology. There will be three presenters from Ireland, Hungary and Canada. The presentations will be preceded by a questionnaire feedback session where the opinions of the participants on the topics of interest will be elicited. The three topics under examination are 1. Prediction of calving, 2. Assessment of neonatal calf vitality and 3. Colostrum management. Each of these topics will be discussed at three workstations, moderated and recorded by delegates and overseen by the workshop presenters. Following this brainstorming session, presentations will be delivered and the outcomes of the three workstations discussed between presenters and delegates. The workshop will conclude with a summary of the key take home messages and recording of the outputs of the workshop for communication to delegate attendees post-conference.

Results

Achieving high-levels of passive immunity is critical to reduce the risk of common calf-hood disease, such as diarrhea and respiratory disease. Successful transfer of passive immunity can be achieved through feeding an adequate quantity of high-quality, clean colostrum quickly after calving. Although new research has identified tweaks that can be made to further enhance colostrum management on dairy farms, the rates of failed transfer of passive immunity remain high. Therefore, it is critical to understand the main barriers of change for calf management on dairy farms and the critical role veterinary practitioners can play. A major barrier to improve calf care is a lack of knowledge regarding best management practices for calf care. As veterinarians are highly trusted to be a primary source of knowledge for dairy farmers, veterinary engagement in calf management can have a tremendous impact. Additional barriers include the prioritization of the milking herd, meaning producers value simple, time-efficient and economical solutions to calf care challenges. Other barriers include farm infrastructure and challenges in employee training. Beyond barriers, it has been identified that producers are motivated by social norms, intrinsic pride, and an obligation to provide good calf care. This may provide insight into why benchmarking has been found to be an important tool to motivate producers. Veterinarians can play a key role in overcoming these barriers and managing programs to motivate change in calf care practices.

Conclusions

Colostrum management has been consistently identified as a major influencer of calf health; however, uptake of optimal colostrum management strategies has been low. Understanding barriers and motivations for change can aid in improving the rates of

passive immunity. Veterinarians play a major role in driving on-farm change and can serve as a means to deliver up-to-date information on colostrum management to farmers.

1491 - Use of injectable mineral and vitamin supplementation and its effects in weight gain and daily weight gain in Nelore grassed calves in South Brazil

Author: JOÃO PAULO LOLLATO MENDES, Caio H. Borges, Angelo Favaro Junior, Nilson Yoshii, Reuel Luiz Gonçalves, Milton Maturana Filho, Gustave Decuadro Hansen

Objectives
The aim off study was assess de weight gain (WG) and average daily gain (ADG) in Nelore calves fed with grass and water *at libitum* and mineral supplementation *per oss* receiving or not injectable mineral and vitamin supplements.
Material and methods
The study was realized in a farm in Amaporã City, located in the Northwest of Paraná State in Brazil The experiment used 315 animals (12.7 ± 2.65 mo old, 282.48 ± 7.90 Kg body weight [BW]) randomized by BW and divided in 2 groups, Treatment Group (G1) that received injectable mineral and vitamin supplementation (KIT ADAPTADOR, Biogénesis Bagó Brazil; 1 mL / 50 Kg L.B.W) and Control Group (G2) did not receive the injectable mineral and vitamin supplementation. The animals were kept at the same group during the hole study. Both groups received oral deworming with Closantel 7,5% (Galgosantel Oral, Biogénesis Bagó Brazil; 1 mL/10 Kg Live body weight). After 36 days (D36), the animals returned to weight management where the G1 received another dose of injectable mineral and vitamin supplementation and 33 days after, totalizing 69 days (D69) from the beginning, the animals were weighted for a final body weight data. Data was analyzed with the software Statistical Analysis System for Windows SAS® version 9.3.
Results
The weight gain in D69 was greater to treatment group (G1 = 50.15 ± 3.30 Kg; G2 = 42.80 ± 3.10 Kg; P=0,01) and the average daily gain in D69 was greater to treatment group too (G1 = 0.760 ± 0.040 Kg/day; G2 = 0.640 ± 0.040 Kg/day; P=0,04).
Conclusions
Thus, the use of injectable mineral and vitamin supplementation (KIT ADAPTADOR, Biogénesis Bagó) was efficient to improve the weight gain and daily weight gain in Nelore calves in pasture and considering the kilograms sales price in Brazil (R$ 9,50, Scot Consultancy, December 20th, 2023) it could impact positively the financial health of the farms by improving the animal performance.

1504 - The Effects of Single Dose of Cabergoline Application During Sudden Drying-off on Milk Leakage, Udder Involution and Mastitis Originating from the Dry Period

Author: Fatih Buyukbudak, Murat Findik

Objectives
This study aims to investigate the effectiveness of a single dose of cabergoline at the beginning of the dry period against milk leakage in cows during the drying-off period, the clinical process of udder involution, and mastitis cases originating from the dry period.
Material and methods
Two study groups were formed from 65 Holstein Friesian cows that were within the last three months of their lactation period with no history of mastitis, had a CMT score of 0 in each mammary quarter, with a milk output of >17 liters/day at the start of the drying-off. Groups were named as cabergoline (GKab) and control (GKon). The GKab group (n=33) received a single i.m. injection of 5.6 mg/5 ml cabergoline (Velactis®, Ceva Santé Animale, France), while the cows in the GKon group (n=32) received a single i.m. injection of %0,9 NaCl (5 ml). Milk leakage and mammary width measurements were performed during drying-off (day 0) and on the 1st, 2nd, 7th, and 15th days. Blood sampling was performed to determine serum PRL (Prolactin) on the 0th, 1st, 7th and 15th days. To evaluate the signs of mastitis originating from the dry period, mammary examination was performed on postpartum day 1, MDI (Mastitis Detection Index) findings, mammary examination, foremilk examination and CMT (California Mastitis Test) were performed on postpartum 4 and 7 days.
Results
The serum PRL levels in GKab and GKon groups were 62.6±125.1 ng/ml and 98.2±153.1 ng/ml ($p<0.045$) at the first day, respectively. Milk leakage was recorded in GKab and GKon as 3.0% (1/33) and 46.9% (15/32) ($p<0.001$) at the 1st day of the drying-off period; 18.2% (6/33) and 56.3% (18/32) ($p=0.002$) at the 2nd day, respectively. Udder measurements were 7.69±1.45 cm and 6.55±1.35 cm ($p=0.002$) at the 0th day, 6.90±2.01 cm and 8.29±1.56 cm ($p=0.001$) at the 1st day, 7.37±1.79 cm and 8.84±1.86 cm ($p=0.002$) at the 2nd day for the GKab and GKon groups respectively. The postpartum mastitis ratios were 3.0% (1/33) and 21.9% (7/32) in the GKab and GKon groups, respectively.
Conclusions
When the data obtained from this study were evaluated, single-dose cabergoline application did not cause any side effects in cows and significantly reduced the incidence of milk leakage. It clinically accelerated mammary involution and showed that it could be a successful management strategy in preventing mastitis cases originating from the dry period.

1510 - Hand-meter portable to monitor metabolic status in ewes under field conditions.

Author: A.J. Alonso-Diez, Jose Ramiro Gonzalez-Montaña , F. Martínez-Pastor, A.E. Hefnawy Abd Elghany, M.A. Blanco-Fernández, I. Sanz-Jerónimo, L.G. Cal-Pereyra, J.L. Benedito Castellote

Objectives

Ovine pregnancy toxaemia is a metabolic disease, due to a negative energy balance, that affects sheep in the last third of gestation, with a marked economic impact due to its high morbidity and mortality rate. It presents with digestive and neuromuscular clinical signs and is characterized by hypoglycaemia, hyperketonaemia, and ketonuria. Although various parameters may appear altered, glucose and ß-hydroxybutyrate (BOHB) allow it to be diagnosed, even without the presence of clinical symptoms.

Given that early diagnosis is key when establishing the necessary measures for its control and prevention, we have evaluated the usefulness of a portable measuring device (GlucoMen areo2K®, Menarini diagnostics) together with adapted test strips.

Material and methods

We have assessed in situ glycemia and BOHB in blood obtained from the jugular vein and auricular vein of sheep in the final gestation period (n=50), and we have compared them with the values obtained in a reference laboratory. Additionally, we have evaluated the suitability of using a blood sample obtained from the auricular vein, as it is a simpler sampling method to be measured using a portable device.

Results

Although there is not always complete agreement between the different measurements made, the portable meter allows an approximation of the blood glucose and ketone body values under field conditions. Furthermore, the results show the suitability of analysing the concentration of both glucose and BOHB in a drop of blood obtained from an ear vein.

Conclusions

The measurement of these parameters will allow early detection of a negative energy balance, detecting in situ sheep with gestation toxaemia or at risk of suffering from it, and thus establish the appropriate control measures.

1511 - Mastitis in sheep flock. Diagnosis and control by somatic cells counting.

Author: Jose Ramiro Gonzalez-Montaña , A.J. Alonso-Diez, A. Fernández-Olivera, M.J. Martín-Alonso, M.E. Alonso de la Varga, M.P. Alonso-Alonso, B. Alegre-Gutiérrez, J.C. Domínguez

Objectives

Clinical Case: In a dairy sheep farm, the somatic cell count (SCC) is a procedure that is as essential as it is common to ensure adequate milk quality. This count is used as an indicator of possible infection in the mammary gland, that is, animals with a high SCC in milk possibly suffer from mastitis, whether clinical or subclinical (Paape et al., 2007, 2001). A high SCC is associated with the loss of milk production and, therefore, with important economic losses, while a low number of somatic cells in milk translates into a correct health status of the animals and greater economic profitability of livestock (Djabri et al., 2002; Harmon, 2001).

A farmer requested our collaboration due to a high SCC in the milk produced, which has a negative impact on the price of the milk from the collecting company.

Material and methods

For this reason, it was necessary to propose a series of control and prevention methods focused on reducing the number of sheep affected by mastitis. These measures will depend on several factors, such as the animal itself, the microorganism involved, the moment of lactation, the degree of involvement of the breast, the production of the sheep, its age, etc. (Aparicio et al., 2001). These factors will be key to choosing how to proceed with each sheep, which traditionally ranges from the elimination of severely affected animals to the use of antibiotic therapy during lactation to drying therapy using intramammary cannulas or the so-called “selective management and/or selective drying” (Bradley et al., 2018; Herrera Mateo, 2018).

Results

For all these reasons, we established an action protocol aimed at

1. Identifying sick animals with clinical mastitis;
2. Identify animals with high somatic cell counts in milk,
3. Establish a treatment and control strategy for sick animals; and
4. Apply a selective treatment in the different batches of animals.

Conclusions

Implementing the indicated measures has had important economic and health repercussions, considerably reducing the SCC in milk, while the amount of milk produced has been maintained. In addition, it has also allowed the cost of treatments to be reduced, avoids unnecessary work for operators, and above all, can maintain an adequate health status of the farm.

1524 - Prevalence of subclinical lung lesions in dairy calves and its impact on average daily gain

Author: Francesco Testa , Stefano Allodi, Giovanni Bufalo

Objectives

Diagnose of subclinical diseases of dairy calf's lung with ultrasound has proven to be really accurate and easy to perform, allowing a better knowledge of risk factors in dairy calf's rearing. Technical services offered by NPM Tech, a feeding company based in Mantua, Northern Italy, include thoracic ultrasound evaluation of a significative sample of calves from birth to 5 months of age. We wanted to verify the accuracy of "Coughing" symptom as a test for the presence of lung lesions and to relate the prevalence of lung lesions to average daily gain in dairy calves.

Material and methods

Ultrasonographic lung consolidations were recorded using the score system proposed by Ollivett in 2016. Respiratory symptoms of the same calves were also recorded using the Calf Health Scoring Chart by the University of Wisconsin-Madison. We analyzed data from 2533 calves in 51 dairy farms from 2020 to 2023 and we related ultrasound results to clinical scores. Using weight data of calves recorded by NPM Tech, we could also relate the prevalence of lung consolidation and the average daily gain of dairy calves in dairy herds where thoracic ultrasound examination was performed. We defined as High Risk Herds the ones were more than 14% of the calves scanned had an ecographic score >2, while we defined as Low Risk Herds the ones were less than 15% of the calves scanned had an ecographic score >2.

Results

We could confirm that “Coughing” symptom has low Sensitivity (19,89%; 15,84%-24,44%) and high Specificity (98,35%; 97,72%-98,84%) as a test for the presence of lung lesions.We could find a significant decrease in average daily gain in High Risk Herds compared with Low Risk Herds in the years of analyses (2020: 0,75 vs 0,82; 2021: 0,76 vs 0,87; 2022: 0,84 vs 0,94; 2023: 0,69 vs 0,85, respectively).

Conclusions

The presence of lung lesions, even If subclinical, is a risk factor for a decreased average daily gain in dairy calves, and the diagnose based on the mere presence of clinical symptoms can lead to underestimate problems that can negatively affect the growth of dairy calves.

1526 - STANDARDIZATION OF MONOCYTE-DERIVED MACROPHAGES CULTURE AND NITRIC OXIDE PRODUCTION IN CATTLE

Author: Bianca Paola Santarosa , Viviani Gomes, Cristina de Oliveira Massoco Salles Gomes

Objectives

Among mononuclear cells, monocytes have heterogeneous functions. They can differentiate into macrophages in tissues, whose main function as an antigen-presentation cell is the recognition, capture, phagocytosis, and microbial activity against antigens by different mechanisms, including the production of nitric oxide (NO). Studies with monocyte-derived macrophages (MDM) cell cultures are scarce in cattle, and NO quantification is generally low. The objective of this study was to describe the producers adopted to cultivate mononuclear cells to the differentiation in macrophages derived from blood monocytes, followed by the quantification of the production of NO, after stimulation with Phorbol Myristate Acetate (PMA) and lipopolysaccharide (LPS) under different concentrations and incubation times between 24h and 72h.

Material and methods

To achieve this, a healthy two-year-old castrated male Holstein bovine was used. A 20mL whole blood sample was collected in a polyethylene syringe containing 0.5mL of 200mM EDTA through jugular vein puncture with prior antiseptic measures. A 0.5mL aliquot was sent for a complete blood count using an automated hematological analyzer (ADVIA2120i®). Peripheral blood mononuclear cells (PBMC) were isolated, and mononuclear cells (MN) were separated by density gradient. After verifying cell viability in a Neubauer chamber using 0.4% Trypan Blue staining, the cell concentration was adjusted to 1×10^7 cells/mL in supplemented DMEM medium (10% inactivated Fetal Bovine Serum, 1% L-glutamine, and 100µg/mL of Gentamicin). Then, 2×10^6 cells/well (200µL volume) were added to a flat-bottom 96-well plate and kept in a CO_2 incubator at 37°C. The next day, the wells were washed with phosphate-buffered saline (PBS) to remove non-adherent cells and lymphocytes. Subsequently, 80% of the supplemented DMEM medium was changed every 48 hours until day 7, when stimuli were applied. The following concentrations of antigens were tested in duplicate: 200 and 400nM PMA; 1µg/mL, 2µg/mL, 5µg/mL, 10µg/mL, and 20µg/mL of LPS. The plate was incubated again for 24 and 72 hours. Finally, the supernatant was removed and frozen at -80°C for subsequent NO measurement using a commercial colorimetric test (Griess method, read at 540 nm on a spectrophotometer).

Results

White blood cell (WBC) counts (9.93×10^3 cells/µL) were within the reference ranges for the species and age group. The monocyte concentration was 0.36×10^3 cells/µL. A total of 523×10^6 live cells were obtained, with 97.94% viability in PBMC. Cell spreading was observed on the fourth day of cell culture, but the morphological change of adherent cells was more evident on the seventh day. The peak of NO concentration produced by cells was observed with 10µg/mL LPS was 17.44mM after 24 hours, and with 400nM PMA, it was 10.26mM after 72 hours. Other stimulus concentrations resulted in NO < 1mM.

Conclusions

It is important to emphasize that to achieve this result, several unsatisfactory pilots were conducted for the detection of NO. The cell culture protocol for functional evaluation of the MDM lineage was based on studies in mice, therefore, it was necessary specific adjustments for bovines. In the literature, there is much divergence regarding cell concentrations, cell culture period, and types of stimuli. In this study, it can be inferred that 10 µg/mL LPS and 400nM PMA were effective in stimulating NO production in MDM with 24 and 72 h of stimulation, respectively. Further research is recommended to standardize the protocol, especially concerning the duration and concentration of stimuli for increased NO production. Additionally, exploring other biomarkers, such as arginase activity, characteristic of alternatively activated macrophages (M2), could provide valuable insights. The seven-day cell culture period proved effective for inducing morphological changes indicative of macrophages, as observed under inverted optical microscopy.

1534 - USE OF WASTE MILK IN PRE-WEANED CALVES FROM BRAZILIAN DAIRY FARMS

Author: Camila Cecília Martin, Larissa Miranda Padilha , Michail Sabino Moroz, Richard Van Vleck Pereira, Viviani Gomes

Objectives
The use of waste milk (WM) as a source in the liquid diet poses biological risks for suckling calves due to potential pathobiotic microorganisms and residual antimicrobials, along with its variable nutritional content. This survey aimed to delineate WM usage practices in the nutritional plan of pre-weaned calves across Brazilian dairy farms (n=1,034), categorized by herd size: small (≤ 20 cows), medium (21 to 70), and large (> 70 cows).
Material and methods
A cross-sectional longitudinal study employed a convenient online questionnaire with snowball sampling for recruitment.
Results
WM was integrated into the pre-weaned diet in 64.0%, 47.22%, and 42.61% of large, medium, and small farms, respectively. Larger properties exhibited 1.52 (95% CI 1.06-2.18) times greater likelihood of feeding pre-weaned calves with WM than smaller ones. Most farms (small: 28.52%, medium: 29.9%, large: 46.9%) initiated WM feeding within the first week of a calf's life without preservation or pasteurization methods (small: 52.92%, medium: 56.49%, large: 49.6%). The exclusive use of WM as the liquid diet throughout the pre-weaned phase was more common in larger properties (75%), followed by medium (25.57%) and small (25.09%) ones. Multiple correspondence analysis (MCA) highlighted associations between larger farms and specific WM practices: early initiation of WM feeding, exclusive use of WM as a diet source, and utilization of WM from cows with mastitis. Conversely, smaller dairy farms were linked to the non-use of WM in liquid diets. A significant majority of large (49.6%), medium (56.49%), and small (52.92%) farms did not implement procedures to reduce or control the microbiological load of WM. Only a small percentage (12.4%, 0.41%, and 0.34% for large, medium, and small farms, respectively) employed pasteurization processes for WM. Additionally, nutritional supplements to enhance WM quality were utilized by 18.76%, 15.1%, and 13.75% of large, medium, and small farms, respectively. Financial reasons were cited by managers and producers as the primary motivation (small: 20.95%, medium: 26.19%, large: 30.60%) for using WM in calf feeding due to its considerable cost in the pre-weaned phase. Some also mentioned convenience (small: 14.1%, medium: 18%, large: 14.8%) and difficulties in finding alternative disposal methods (small: 2.3%, medium: 4.5%, large: 13.8%) as additional factors. Concerning the disposal of discarded milk, large properties reported the use of manure pits (34.1%) and sewage networks (26.0%). Conversely, a higher proportion of medium (27.01%) and small (25.77%) properties utilized all produced WM without disposal. Additionally, 18.21% of small farms used excess WM to feed other animals (e.g., dogs, cats, pigs), a practice reported in only 10.10% of medium and 4.65% of large properties.
Conclusions
MCA analysis unveiled associations between smaller farms and using WM for other animal species and challenges in discarding milk with antimicrobial residues. In contrast, larger farms were associated with WM disposal in manure pits. This study offers insights to inform future research and guidelines for producers and regulatory agencies.

1535 - COLOSTRUM MANAGEMENT PRACTICES IN PRE-WEANED CALVES IN BRAZILIAN DAIRY PRODUCTION SYSTEMS

Author: Camila Cecília Martin, Larissa Miranda Padilha , Michail Sabino Moroz, Richard Van Vleck Pereira, Viviani Gomes

Objectives
Efficient calf management practices are essential in achieving mortality, morbidity, and long-term productivity goals. This survey aimed to explore colostrum management practices adopted in dairy-raising systems located in different states of Brazil.
Material and methods
A cross-sectional longitudinal study using an online questionnaire gathered responses from 1,034 participants.
Results
Regarding colostrum management, only 3.09%, 12.78%, and 56.59% of small, medium, and large properties, respectively, assessed colostrum's immunological quality before feeding calves. Large farms were 40.85% more likely (95% CI 20.12-82.9) to employ methods to evaluate colostrum quality compared to smaller properties. The primary evaluation method cited by producers was the Brix refractometer (small 2.3%, medium 11.7%, large 42%), followed by the colostrometer (small 0.8%, medium 1.1%, large 14.5%). Multiple correspondence analysis (MCA) revealed an association between large farms and regular colostrum quality assessment practices, using diverse evaluation methods. Conversely, smaller farms were linked to the absence of any method to assess colostrum immunological quality. The predominant method of colostrum feeding was via bottle, irrespective of property size. Natural suckling occurred in 34.71% of small dairy properties, while only 8.14% of large farms reported this practice. Small properties were 6.08 times more likely (95% CI 3.66-10.1) to allow natural colostrum intake than larger farms. Larger properties provided larger colostrum volumes (32.17%, 3-4 liters) compared to medium-sized ones (32.37%, 2-3 liters). Additionally, 48.11% of small properties reported *ad libitum* colostrum ingestion during the first feeding, posing a 4.75 times higher risk (95% CI 3.17-7.06) compared to larger farms, where the volume intake was controlled. The use of frozen colostrum was more prevalent in larger properties (52.71%) than in small (13.75%) and medium (22.47%) properties. Notably, most large properties (30.23%) provided colostrum between two and four hours after birth, while a significant number of medium properties (29.07%) were unsure of the ideal timing for the initial colostrum feeding. In small properties, producers reported natural ingestion of colostrum directly from dams (36.08%). Offering a second colostrum feeding between six and 12 hours post-birth was more common in larger farms (24.03%) compared to medium (15.2%) and small (8.0%) properties. MCA analysis indicated an association between larger farms and the adoption of gold-standard colostrum management practices. Differences were noted in colostrum supplement and substitute usage among property sizes, with larger herds (20.16%) more frequently using these products compared to small (2.41%) and medium (6.8%) properties. Large properties were 10.26 times more likely (95% CI 4.57-23.04) to utilize colostrum supplements than small ones, while medium properties showed 2.99 times higher chances (95% CI 1.31-6.85). Pasteurization practices did not significantly differ among property sizes. However, colostrum pasteurization was more prevalent in high milk production farms. It was 7.05

times more likely (95% CI 1.56-31.79) to occur in large properties compared to small ones, while no differences were found in medium-sized properties.

Conclusions

These findings illustrated that property size influences calf management practices, notably in colostrum management, with larger properties exhibiting better indicators. Therefore, emphasizing employee training and altering producer perceptions, especially in smaller properties, becomes crucial. Veterinary guidance and monitoring are essential to minimize calf morbidity, mitigate economic losses, and ensure superior post-weaning adaptation and performance.

1566 - Hierarchy of cows: association between hierarchy, rumination-time, rumen pH and production of dairy cows during early lactation

Author: Mari Hovinen , Irmeli Kärkkäinen, Tuomo Kokkonen

Objectives

During transition period calf is born, milk production starts and feed changes to high-energy roughage and concentrates with high protein and starch content. Cows become prone to stress and metabolic and infective diseases. Coping depends on the facilities offered, for example length of the feeding fence and group size. Cows high in hierarchy might do better in the battle of resources. This could potentially affect rumen pH, rumination time and milk production. The aim was to study the associations between hierarchy of dairy cows, rumen pH, rumination time and milk production during transition period until 100 DIM.

Material and methods

Reticular pH of 20 cows was measured in a free-stall dairy barn. Oral boluses (SmaXtech animal care®, Graz, Austria) measured pH of the reticulum and a rumination index using wireless, online technique. The boluses were inserted 14 days before the expected calving, when cows were in a close-up pen with free stalls. After calving, cows were put either in a fresh-cow pen with 93 cm feeding fence per cow, or in a milking compartment with ~53 cm feeding fence. Automatic milking systems measured milk production and cow weight.

Hierarchy of cows was determined in two occasions in the milking compartment (first from 33 to 78 DIM and second from 94 to 139 DIM). At this point, 9 first calvers and 8 older cows remained in the herd. The observation time was 12 and 8 hours, using video recording as an aid. An index of success in competitive interactions (Galindo and Broom, 2000) was calculated for each cow by dividing the number of replacements by the total number of interactions (either being replaced from the feeding fence or replacing another cow from it).

The cows were fed grass-silage based mixed-ration supplemented with concentrates up to 7 kg from the milking robot. The proportion of concentrates was ~55% in the whole ration and proportion of fiber from roughage was ~340 g/kg dry matter. Dry cows were fed total mixed ration consisting of grass silage and hay complemented with 250 g/d of concentrates. Associations between hierarchy, pH, rumination, and milk production were analyzed using Stata/MP 16.0.

Results

Eleven cows belonged to low-success group (LH) and 6 cows were higher in hierarchy (HH). This result correlated well with age and weight of the cows; all first-calvers and 2 older cows were LH.

Cows experienced a decline in reticular pH after calving. The pH was normalized by 10 DIM to 6,9 (HH) and 6,87 (LH) compared to 6,7 before calving. The nadir seemed lower for HH cows (mean 6,4 and mean of the lowest values at 2 DIM 6,16) compared to LH cows (6,6 and 6,3, respectively). After 15 DIM, milk yield of HH cows was 44,5 kg milk/day, achieving a peak in 25 days and starting to decline after 50 DIM. LH cows achieved a stable level of 34,5 kg milk 3 weeks *post partum*, with no peak in yield. After calving, HH cows weighed 636 (538 – 706) kg and lost ~75 kg during 100 days. LH cows weighed 536 (463 – 577) kg, and lost ~60kg during 75 days. However, HH cows gained

weight at first 40 DIM, while LH cows lost weight already during the first week *post partum*. There were no big differences in rumination time between the groups (both roughly 600 min/day), although the nadir reached 325 min for HH cows at 1 DIM, compared to 360 min in LH cows.

Conclusions

In modern dairy farming with high level of fiber in feed and several feeding times it is possible to keep rumen pH at optimal level and rumination time high. However, there is a risk of a short period of subacute rumen acidosis immediately after calving specially with dominant cows. They are older, heavier cows, and they have higher milk yield with higher production peak than mainly primiparous cows low in hierarchy. In this study, the dominant cows lost weight until 100 DIM and further, more than recommended.

References

Galindo F and Broom D. 2000. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. Veterinary Science 69, 75–79.

1568 - Bacterial microbiome of individual cow and bulk-tank milk samples with low and high somatic-cell count

Author: Viktoria Neubauer , Franz-Ferdinand Roch, Monika Dzieciol, Martin Wagner

Objectives

Mastitis is an economically detrimental disease because of lower production, reduced milk quality, potential infectious agents, and often labour-intensive treatment. After fertility, mastitis is the second most probable reason for culling dairy cows worldwide. Among the different forms of mastitis, subclinical mastitis poses a particular challenge to veterinarians and farmers due to its complex or unknown pathogenesis. In this study, we aimed to investigate the milk microbiome to gain knowledge about changes in the commensal udder-milk bacteria with increasing somatic-cell count (SCC). Additionally, we evaluated the potential of bulk-tank milk to reflect the milk microbiome of the herd, to use this sample as a future subclinical-mastitis diagnostic tool.

Material and methods

During two sampling events, one in winter and one in summer, milk samples were collected from eight Austrian dairy farms (average of 36 milking cows per herd). The farms were categorized into four low SCC farms (LSCC; <200,000 cells/mL in bulk-tank milk (BTM)) and four high SCC farms (HSCC; >200,000 cells/mL), based on their bulk-tank SCC during the observation period. None of the herds exceeded the 400,000 cells threshold for shipment. We took BTM and aseptic collective-milk samples (CM) from an average of ten cows per farm and sampling event. California-Mastitis-Test (CMT) was performed for all sampled cows. From a total of 16 BTM and 162 CM samples, we extracted bacterial gDNA and sent it for Illumina MiSeq amplicon sequencing, targeting the V3-V4 region of the 16S rRNA gene. We trimmed the reads using Trimmomatic and further processed them in QIIME2, including taxonomic classification using the GreenGenes database. To ensure data quality, we excluded samples with fewer than 1,000 reads or less than 99% Good's coverage. This resulted in a final set of 16 BTM and 151 CM samples for detailed analysis. We used coverage-based rarefaction to calculate Shannon and Simpson indices, assessing alpha-diversity. We measured beta-diversity using Bray-Curtis dissimilarity. All rarefaction, statistical analyses, diversity parameter calculations, and the creation of plots were performed and generated using R.

Results

SCC values of the analysed CM samples ranged from 9×10^3– 8×10^6 cells/mL. Beta-diversity analysis resulted in clusters for sampling event and farm. For the winter sampling, all but one BTM sample clustered together with the corresponding CM samples. The BTM samples from the summer sampling formed one cluster apart from all the other samples. There was no clustering according to SCC groups. Diversity parameters showed numerically higher alpha-diversity during the winter sampling and differences between the farms. On farm level, CM samples on HSCC farms had higher relative abundance of the genera *Corynebacterium* and *Streptococcus*, and a lower relative abundance of *Staphylococcus*. On individual cow level, *Streptococcus*, *Serratia*, *Pseudomonas*, and the uncultured genus JC017 showed an increasing relative abundance with higher SCC. These results also applied to the CMT test results. BTM samples contained high abundances of *Acinetobacter*, *Aerococcus*, *Chryseobacterium*, *Lactococcus*, and *Leuconostoc,* which is in contrast to the CM samples, where those

genera were low or not found. All other genera that were present in the CM samples also occurred in the BTM samples.

Conclusions

Sampling events and season have a significant impact on the microbiome in individual cows and the bulk-tank milk. Therefore, it is essential to consider these factors during sample collection and data interpretation. There are trends in this sample set that the bacterial community changes with increasing SCC, without showing clinical mastitis. There is good evidence that the prediction of a subclinical-infection status of the herd will be possible using the bulk tank milk, but further investigations are needed.

1578 - METABOLIC STRESS IN HOLSTEIN COWS SUPPLEMENTED WITH INJECTABLE MINERALS IN THE TRANSITION PERIOD

Author: Raquel de Sousa Marques, Clara Satsuki Mori, Bruno Gonzalez, Luc Durel, Bruno Sivieri de Lima, Viviani Gomes

Objectives

During the transition period, excessive mobilization of adipose tissue can lead to immune dysregulation and cause oxidative and metabolic stress in dairy cows. To study this, an injectable mineral supplementation (ITMS) program was implemented, and its effect on oxidative stress, IgG dynamics, and lipid metabolism was evaluated in order to determine the potential benefits.

Material and methods

A study was carried out in a dairy farm situated in São Pedro, São Paulo, Brazil. The farm had around 400 lactating Holstein cows with an average milk yield of 35.0 L/animal/day. Close-up and fresh cow diets were formulated by the farm's nutritionist and met the recommendations of the NRC. Healthy cows were enrolled in the study 30 days before calving, and allocated to either the non-supplemented (NIMS, n=32) or the supplemented group (IMS, n=34). Supplemented cows received three injections (10 mL) of a multi-mineral supplement by intramuscular route 30 to 20 days before calving, at calving, and 11-17 days after parturition. This supplement (FOSFOSAL®, Virbac Brasil, SP, Brasil) was made of sodium glycerophosphate (5.5H2O 14 g), monosodium phosphate (20.1 g), copper chloride (0.4 g), potassium chloride (0.6 g), magnesium chloride (2.5 g), and sodium selenite (0.24 g), in water (100 mL). Cows were evaluated during and after pregnancy: three weeks prepartum (M-3), two weeks (M-2), one week prepatum (M-1), delivery week (M0), one week postpartum (M+1), two and three weeks later (M+2, M+3). Heparinized blood samples (10mL) were collected to determine the total anti-oxidant status (TAS) and serum lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS) and glutathione peroxidase (GPx). EDTA tubes (4mL) were used for the determination of reduced glutathione (GSH). Samples for measuring haptoglobin, IgG, and metabolic biomarkers were collected by using a clot-activator tube to obtain blood serum. IgG concentrations were measured using an in-house sandwich ELISA assay. The concentration of haptoglobin was determined by its ability to bind hemoglobin by using spectrophotometry. BHB and NEFA were determined by using commercial test kits. Data from cows were analyzed within each category (primiparous or multiparous) and in the total population. The MIXED-model procedure determined the effect of treatment, time, and time*treatment interaction. Interactions between treatment and time points were highlighted using a Student's t-test for each prepartum and postpartum period. These interactions were considered significant when $p \leq 0,05$.

Results

In primiparous cows, the effect of treatment was detected for the following variables: IgG (NIMS=27.1±1.5 vs. IMS=33.3±1.9 g/L, P=0.004) and glucose (NIMS=62.2±0.9 vs. IMS=66.3±1.8 mg/L, P=0.049). Effect of time was detected for TBARS, TAS, IgG, haptoglobin, and all metabolic biomarkers (P<0.008), except total protein and NEFA. None of the variables in primiparous animals were found to have a significant interaction with time. The following variables showed a detectable effect of treatment in multiparous cows: GPx (NIMS= 801.90±34.68 vs. IMS=940.73±29.96 UI/g, P= 0.025), IgG

(NIMS=28.0±1.4 vs. IMS =36.87±1.69 g/L, P=0.003), haptoglobin (NIMS=6.15±0.65 vs. IMS=4.32±0.3 mg/mL, P=0.030) and glucose (NIMS=60.3±0.8 vs. IMS=62.9±1.1 mg/L, P=0.067). Effect of time was detected for TBARS, TAS, IgG, haptoglobin, and all metabolic biomarkers (P<0.008), except total protein and NEFA. Interaction group*time was detected for TBARS (P=0.038), TAS (P=0.050), and BHB (P=?). NIMS had a high concentration of TBARS on M+1, while TAS was higher on week +2 and week +3 in the IMS group than in NIMS. Considering the overall population, the mineral supplementation had a detectable effect the GSH concentration (NIMS = 3.44±0.22 vs. IMS = 3.7±0.18 mg/mL, P=0.050), IgG levels (NIMS = 27.68±1.05 vs. IMS = 35.63±1.3 g/L, P<0.001) and haptoglobin (NIMS = 5.84±0.42 vs. IMS = 4.21±0.23 mg/mL, P< 0.001). Effect of time was detected for all variables. Interaction treatment group*time was detected for IgG detecting higher concentrations in the IMS group for all weeks of the transition period compared with NIMS cows.

Conclusions

In the conditions of this study, injectable mineral supplementation was found to be effective in improving the cows' immunity. An anti-inflammatory profile and higher serum IgG concentrations characterized this. Additionally, the supplementation program was also found to reduce lipid metabolism and oxidative stress, which is a positive finding for the overall health of the cows.

1584 - Vertical dam-neonate calf transfer of gut microbiota in the early of life

Author: Viviani Gomes , Christian Hoffmann, Daniela Irlanda Castro-Tárdon, Fernanda Carolina Ramos dos Santos, David John Hurley

Objectives

The intestinal microbiota plays an important role in the immune system, metabolism and development of newborn calves. In cattle, precisely how the neonatal gut bacterial microbiome is initially populated is not well understood. To better understand how the maternal sources influences the acquisition of the calves GIT microbiota, we studied the relationship among the microbiota from dams to the GIT of 20 Holstein young heifers in the first 28 days of life.

Material and methods

It was included 20 Holstein cows and their offsprings. Dams were milked in the maternity pen. Mammary gland was cleaned and disinfected and colostrum samples per quarter were harvested by manual milking in sterile tubes. Samples were pooled by dams to perform the cells isolation. Cellular fraction was preserved between washes, and suspended in 5 mL of sterile PBS, stored at -80ºC freezer. Vaginal secretion was obtained close to the cervix using a long swab covered with a plastic sheath, and fecal samples were harvested by using dry swabs, stored at -80ºC freezer. Calves were fed by using fresh colostrum on the 1st day, followed by the administration of six liters of milk replacer per day containing 14% of total solids. Water and starter were offered *ad libitum*. Fecal swabs of calves were harvested on days 1 (before colostrum intake), 7, 14, 21 and 28. Any calves received oral or systemic antimicrobial through the study. Total DNA was extracted and the sequencing library preparation was carried out in a two-step PCR protocol, following Illumina's process. The 16S rRNA sequences obtained from the microbiome were analyzed using the software QIIME. All subsequent analyses were performed with R version 3.6.1 and RStudio version 3.6.1 statistical programs using phyloseq, vegan, gplots, ggplot2 and qiimer.

Results

The most of newborns had fecal score normal (0 and 1) through the study. Among maternal samples, the highest α-diversity and richness was detected in fecal samples, followed by vaginal secretion. Colostrum samples had the lowest α-diversity and richness values. Neonates had lower fecal diversity and richness in comparison with the fecal samples from their dams. α-diversity and richness in neonates' fecal samples were very similar, except for the values detected on day 7 of life. Looking at the presence or absent of OTUs by Weighted-UniFrac, it was possible to detect Bray-Curtis (BC) similarity between meconium, colostrum and vaginal secretion. It was detected high distance between fecal samples from d3 and d7 from calves and fecal samples from its dams. These distance decrease through the neonatal period, and in day 28 of life, samples from calves evolute in direction to the maternal community profile. Weight-Unifrac was performed to analysis the relative abundance of species shared between samples. Meconium and fecal samples in the early life of calves until day 7 where very similar maternal sources, and the distance between dam and offspring increased according to the calves development in the neonatal period. Sources of maternal samples and possible contamination on the calves raising system were assessed using the Source

Tracker model. The sources included all maternal samples, bedding from maternity pen, milk replacer and water drinking from buckets. The Source Tracker analysis indicated that the microorganisms were significantly correlated to maternal sources (colostrum and vaginal secretion) from day 1 up to day 7. On the other hand, the unknown source (probably environment) had a gradual increase of correlation with fecal samples from calves from day 3 up to d28. Fecal samples from dams seem not to an important source of bacterias for calves. Bdding from maternity is a source of bacterias on meconium, while diluted milk replacer seem to have influence on fecal samples. In general, clusters show similarity between calves according to age. Meconium presented low number of OTUs. The most abundante bacteria group on day 1 (meconium) was Proteobacteria filo_Enterobacter, followed by Firmicutes philo_Streptococcus. On day 3, Proteobacteria_Escherichia-Shiguela was the most predominant bacteria group; on day 7 was Firmicutes philo_Bifidobacterium and Lactobacillus; on day 14 was Fusobacteria_Fusobacterium; on day 28 was Bacterioidetes_Bacteroides and Firmicutes_Faecaliumbacterium.

Conclusions

Dams are source of bacterias to their offspring in the first three days of life, however external factors unknown had intense influence on gut microbiota across the neonate period.

1587 - Ozone therapy in dairy goats infected by Small Ruminant Lentiviruses improves clinical and ultrasound parameters

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Objectives

Small ruminant lentiviruses (SRLV) are chronic and progressive diseases that compromise the production, health and animal welfare. Due to the lack of cure, vaccines or any effective treatments against lentiviruses, ozone therapy, a treatment that uses a mixture of O3/O2 gases, emerges as a possible tool for clinical intervention. Therefore, this study aimed to evaluate its therapeutic effect on different clinical and joint ultrasound indices in dairy goats naturally infected by SRLV.

Material and methods

This study was approved by an ethical committee for animal use (number: 5810141022). A total of 42 adult Saanen goats (4.4 ± 1.4 years old; 2.6 ± 0.2 body condition score [BCS]; 60.1 ± 10.4 kg) with chronic clinical signs of arthritis and weight loss were used after confirmation of infection (PCR) and/or seropositivity (Western Blot) to SRLV. Animals were allocated to three experimental groups: $G_{oxygen/ozone}$ (n=14) treated with ozone therapy by rectal and intra-articular routes, once a week and every 30 days, respectively, for 90 days; G_{oxygen} (n=14) treated with oxygen therapy by same routes and periods mentioned previously; and $G_{control}$ (n=14) which received no treatment throughout this period. A therapeutic ozone dose of 0.05 mg/kg was adopted. Syringes (60 mL) were connected to the generator output where the O2/O3 gas mixture was collected using the following parameters: O2 flow = 0.125 L/min and an ozone concentration of 22 µg/mL. The following formula was used to calculate the final gas volume (O3 VOL) for each animal: O3 VOL (mL)= (O3 QTY [mg] / O3 CONCENTRATION [µg/mL]) x 1000. Being O3 QTY (mg) = DOSE (mg/kg) x LIVE WEIGHT(Kg). The O3 VOL was updated every month based on the live weight of each animal. A siliconized syringe containing 5 mL of medicinal O2 or O2/O3 gas mixture was used for the intra-articular procedure carried out after carpal antisepsis. All groups were monitored for 90 days. Every month, all goats underwent a clinical examination, collection of production indices and carpal ultrasound. For comparison among groups, analysis of variance (ANOVA) was used followed by Tukey's test at a significance level of 95%.

Results

There was a treatment effect ($p \leq 0.05$) for productive (BCS and live weight) and clinical indices (carpal joint index, biometric indices and carpal volume). $G_{oxygen/ozone}$ showed a greater BCS when compared to $G_{control}$, and G_{oxygen} had a score equal to other groups (2.7±0.4 ≠ 2.5±0.3 = 2.6±0.4 BCS). $G_{oxygen/ozone}$ had a greater live weight compared to $G_{control}$, even though equal to G_{oxygen} (62.0±9.8 ≠ 57.2±9.5 = 61.2±11.3 kg). In clinical evaluations, G_{oxygen} and $G_{oxygen/ozone}$ obtained lower carpal joint indices when compared to $G_{control}$ (8.2±1.0 = 8.2±1.3 ≠ 8.7±1.0 cm). In biometric assessments of the carpus (height, width and depth), G_{oxygen} demonstrated a greater reduction ($p \leq 0.05$) when compared to other groups. In all volumes studied (elipse, lambert and cylinder), the G_{oxygen} also obtained lower carpal volume values when compared to other groups. There

was also a statistical significant improvement ($p \leq 0.05$) for joint ultrasound indices (joint space, sagittal and transverse section of the carpus, carpal volumes, distance from the joint capsule to the subchondral space, area and perimeter of the carpal lesion). Such indices obtained lower values in one or both treated groups ($G_{oxygen/Gozone}$ and G_{ozone}) when compared to $G_{control}$. The values obtained among groups for the distance from the joint capsule to the subchondral space (8.6±3.2 = 8.7±3.7 ≠ 10.9±4.3 mm), area (156.2±69.4 = 153.7±68.1 ≠ 239.0±89.3 mm) and perimeter (81.1±16.4 = 81.6±15.7 ≠ 101.7±15.9 mm) of the carpal lesion are highlighted. Likewise, there was interaction between treatment and time ($p \leq 0.05$) for ultrasound parameters of the distance from the joint capsule to the subchondral space, joint area and perimeter of the carpal lesion. In these, G_{oxygen} and $G_{oxygen/ozone}$ presented lower values on D60 and D90 when compared to $G_{control}$. Regarding the area of carpal lesion, there was a drop of 20% and 25% for G_{oxygen} and $G_{oxygen/ozone}$, respectively, when compared to the 37% increase in $G_{control}$ from D0 to D90.

Conclusions

Although $G_{oxygen/ozone}$ obtained some better results in productive indexes, only the oxygen therapy (G_{oxygen}) showed to be equally suitable when compared to ozone therapy targeting carpal arthritis. Therefore, both therapies, under current conditions, can be safely used to improve joint clinical signs and production rates in goats infected with SRLV.

1593 - Quantitative ultrasound attributes for spleen, liver and kidney in healthy hair sheep

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Objectives

Ultrasonography (US) is a non-invasive and practical tool widely used for diagnosis of numerous ruminant disorders. Despite advantages offered, the US has limitations regarding image interpretation, which may differ according to examiner's practice. In this sense, computerized image analysis can express objective measures of the echogenic properties from biological tissues. Therefore, it was aimed to describe quantitative ultrasound attributes for spleen, liver and kidney in healthy hair ewes.

Material and methods

This study was approved by an ethical committee for animal use (number: 2919170821). To attest animals' health, a complete clinical examination and laboratory tests for whole blood count, renal and liver biochemistry were performed. In sequence, the spleen, liver, and kidneys of 35 adult Santa Inês ewes (4.9 ± 0.7 years old; 49.2 ± 8.7 kg; and 3.0 ± 0.1 body condition score) kept under intensive system were evaluated by transabdominal ultrasonography. US evaluations were performed using portable B-mode equipment with a 7.5 and 5.0 MHz linear and convex transducer, respectively. Throughout all US scans, animals were maintained in standing position contained by a trained person. A general ultrasound examination of the spleen and liver were carried out in each intercostal space (ICE) with the transducer held parallel to ribs, beginning from dorsal-ventral and caudo-cranial directions. To assess kidneys, the right flank was divided into two imaginary quadrants (Q2 and Q1) and the area of the 12^{th} and 11^{th} ICE was also demarcated. Both kidneys were examined in longitudinal sections with the transducer maintained parallel to its longitudinal axis and in cross sections with the transducer maintained perpendicular to the longitudinal kidney axis. For quantitative US evaluation, it was performed a computerized image analysis using a commercial software. Mean numerical pixel values, pixel heterogeneity and minimum and maximum pixel values were measured by selecting a region of interest (ROI) in rectangular format with base and height dimensions of 300 x 90 respectively for splenic and hepatic parenchyma analysis. For the renal cortical and medullar region, the ROI was drawn in the most homogeneous region and close to the focus tool. A grayscale histogram (HEC) for each assessed tissue was produced. For comparison between quantitative ultrasound attributes, analysis of variance (ANOVA) was used followed by Tukey's test at a significance level of 95%.

Results

There was a transducer effect on the comparison between the number and heterogeneity of pixels of the organs evaluated ($p \leq 0.05$). The renal cortex and liver presented a different number of pixels according to the transducer adopted (Spleen – 93.7±15.6 = 93.6±12.4; Liver – 79.9±11.7 ≠ 84.8±9.6; Right renal cortical – 93.7±11.6 ≠ 68.9±12.6; Left renal cortical – 104.8±15.9 ≠ 78.0±11.9; respectively for convex and linear transducer). All organs showed different pixel heterogeneity according to the transducer adopted (Spleen – 12.1±2.7 ≠ 10.5±1.4; Liver – 10.7±2.1 ≠ 11.9±2.2; Right

renal cortical – 8.9±1.9 ≠ 7.0±1.9; Left renal cortical – 8.8±1.9 ≠ 6.7±1.9; respectively for convex and linear transducer). When comparing the right and left renal cortex, it was also found that, regardless of the transducer, the left side obtained higher values for the number of pixels when compared to the right side ($p \leq 0.05$). There was no side effect for the renal medullary obtained only by the convex transducer (mean number of pixels – 67.7±9.9; mean pixel heterogeneity – 4.9±1.5; $p > 0.05$). There was also a transducer effect on the comparison of the number and pixel heterogeneity among the organs studied ($p \leq 0.05$). Considering the convex transducer, the number of pixels in the left renal cortical was higher than others, with the spleen and right renal cortical without differences and the liver being the lowest. Considering the linear transducer, the number of pixels in the spleen was the highest, followed by the liver and left renal cortical equally. The right renal cortical had the lowest values. Finally, the pixel heterogeneity in the spleen and liver, regardless of the transducer or combining both, were higher than that obtained to kidneys.

Conclusions

Through current outcomes, it was possible to establish quantitative ultrasound attributes for the liver, spleen and kidneys in sheep. Such data can reduce the operator subjectivity for definition of echogenicity and echotexture criteria in the clinical routine or research purposes.

1603 - Evaluation of blood urea nitrogen as a new hematologic indicator associated with growth and development in neonatal Hanwoo calves

Author: Youngjun Kim

Objectives
In immunologically naïve neonatal calves, the intake of colostrum within 24 -36 h after birth is responsible for the passive immunity of the calves during their first 3 weeks of life. However, colostrum is important from both immunological and non-immunological perspectives. In this study, we aimed to develop a hematological diagnostic tool for newborn Hanwoo calves to diagnose failure of passive transfer (FPT), as well as for calf growth and development, including average daily gain (ADG) and morbidity. Blood was collected at 12 -24 h of age, and a complete blood count, serum biochemical profile, and blood gas analysis were performed. Moreover, the number of treatments at weaning (up to 3 months of age) was examined, and the body weight at birth, 3 months, and 6 months of age were recorded. From the total of 90 blood samples collected, the overall prevalence of FPT was 24.4% (22/90) in Hanwoo calves. The total serum protein and blood urea nitrogen (BUN) both had significant correlations with the albumin, globulin, albumin/globulin, creatinine, gamma-glutamyl peptidase (GGT), glucose, and total bilirubin. Of note, only BUN was correlated with the ADG. Regarding the receiver operator characteristic curve, BUN was statistically significant as a diagnostic tool for ADG with a 0.374 area under the curve (95% confidence interval: 0.254 -0.494; $P < 0.05$), and the optimal cut point was 7.3 mg/dL (sensitivity 0.65; specificity 0.64). The BUN < 7.3 mg/dL and BUN > 7.3 mg/dL groups showed significant differences in the results for albumin, globulin, albumin/globulin, creatinine, GGT, lymphocytes, and total bilirubin, which was similar to the comparison between the FPT and adaptive passive transfer (APT) groups. The ADG at 0 -3 months and 0 -6 months had a lower morbidity in the BUN < 7.3 mg/dL group. When comparing the ADG and morbidity among the FPT, APT (BUN < 7.3 mg/dL), and APT (BUN > 7.3 mg/dL) groups, the APT (BUN < 7.3 mg/dL) had the highest ADG ($P < 0.01$) and lowest morbidity. Therefore, in addition to the diagnosis of FPT, the analysis of BUN is suggested as a new diagnostic tool for the growth and development of calves.
Material and methods
This study is under review (Journal of dairy science)
Results
This study is under review (Journal of dairy science)
Conclusions
This study is under review (Journal of dairy science)

1607 - Effect of Saccharomyces boulardii CNCM I-1079 on fecal microbiota of preweaned Japanese black calves: A field trial

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Objectives
Previous studies (Mori et al., 2022, 2023) have indicated that supplementation with the probiotic strain *Saccharomyces boulardii* CNCM I-1079 positively influenced vaccine response in young Japanese black calves. While this effect may be linked to alterations in the lower gastrointestinal tract microbiota, how this probiotic specifically impacts the microbiota of Japanese black calves remains unclear. This study aimed to examine the effects of supplementation with *S. boulardii* CNCM I-1079 on the fecal microbiota of preweaned Japanese black calves.
Material and methods
Twenty-five healthy young Japanese black calves enrolled in a previous study (Mori et al., 2023) were selected. All calves were born in the experimental farm and were grown with their dams from birth to 2 weeks of age. After dam–calf separation, *S. boulardii*-group (SB group) calves (n = 15) were fed 2.0×10^{10} CFU/day of *S. boulardii* CNCM I-1079 ("ProTernative," Lallemand-Biotech Co., Ltd., Tokyo) in a milk replacer ("Everymilk," NOSAN Corporation, Yokohama), while the control group calves (n = 10) were fed milk replacer without *S. boulardii*. All calves foraged freely for roughage and concentrate. Fecal samples were collected at 0, 3, 6, and 9 weeks of age, and the V4 region of 16S rRNA was sequenced. Analysis was performed using QIIME2. Alpha and beta diversities were analyzed, along with differential enrichment by analysis of composition of microbiomes (ANCOM) and Linear discriminant analysis Effect Size (LEfSe) analysis.
Results
Calves supplemented with *S. boulardii* CNCM I-1079 exhibited significantly higher alpha diversity (Shannon index, observed features, and Faith's Phylogenetic Diversity) at 9 weeks of age ($p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively). Furthermore, variations in beta diversity (Unweighted Unifrac distances) were noted at 6 and 9 weeks of age ($p < 0.05$ and $p < 0.10$, respectively). ANCOM analysis indicated that SB group calves had elevated levels of *Bacilli*_RF39, which is estimated to produce acetic acid, at 9 weeks of age compared to the control group (W = 32). The abundance of *Lactobacillaceae* in the SB group at 9 weeks was lower than at 6 weeks (W = 23). LEfSe analysis revealed higher levels of *Oscillospiraceae* at 3 weeks and *Bacilli*_RF39 and *Atopobiaceae* at 9 weeks in the SB group compared to the control group. At 6 weeks, the control group exhibited higher abundances of *Muribaculaceae* and *Anaerovoracaceae*. In the SB group, bacteria reported to produce short-chain fatty acids, including *Bacilli*_RF39, were more abundant at 9 weeks than at 6 weeks, along with a higher level of *Methanobacteriaceae*. Conversely, in the control group, the abundance of *Bacilli*_RF39 at 9 weeks was lower than at 6 weeks, with only *Methanobacteriaceae* being more abundant at 9 weeks than at 6 weeks.

Conclusions
Preweaned Japanese black calves supplemented with *S. boulardii* CNCM I-1079 showed higher alpha diversity, different beta diversity, and high levels of bacteria reported to

produce short-chain fatty acids. These findings suggest that *S. boulardii* CNCM I-1079 could increase the number of bacteria producing short-chain fatty acids.

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1630 - Surgical management, diagnosis of legs traumatic and tumor-like disorders associated with housing discomfort in Holstein dairy cows.

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Objectives

The objectives of this study were to evaluate the incidence of the lower legs traumatic and tumor-like disorders associated with housing discomfort in Holstein dairy cows and to evaluate the efficiency of surgical ablation. The excised tissue was submitted for histopathological exam.The most common anatomical location of these disorders are the hocks(tarsal cellulitis) and hoofs(cracks and secondary tumor-like growth) This tarsal cellulitis described in the veterinary literature as tarsal bursitis or tarsal periarthritis, is a chronic cellulitis of the epidermis, dermis and subcutaneous tissue of the hock joint, with unilateral or bilateral localization, commonly on lateral surface. Clinical signs range from a small area of hair loss and hyperkeratosic area to open injuries, with diffuse tumefaction of the area, as a growth, pain just in advanced stages and in some cases, the entire joint are affected, complicate with infection and occurrence of a chronic fistulous secretion.The acropodial traumatic and tumor-like disorders include the well known interdigital hyperplasia and hoof orizontal and vertical cracks with secondary soft tissue proliferation. Prevention of these disorders may include proper freestall design, deep bedding, proper hoof trimming and selecting cows for normal hock conformation.

Material and methods

The study were performed on a dairy Holstein farm with 240 milking cows, with freestall housing, using as a bedding straw and sawdust and mattresses(rubber). By clinical exammination we appreciated the presence of local changes (skin erosion, hair loss, tumefaction with firm tissue or fluctuance, presence of fistulas). Assessment of clinical modifications were completed by morphometric measurement of the hock(tarsal) circumference.The acropodial disorders included traumatic traumatic and tumor-like disorders feasible to surgical excision).The high genetic cows values with an severe enlargement of the tarsal region were selected for surgical ablation. General anesthesia was achieved by administration of Xylazine(Narcoxyl 2, 0.2 mg/kg) in combination with Buthorphanol(Butomidor, 0,05mg/kg). General anesthesia was supplemented by local analgesia with articain/epinefrin(UltracainD-S). Cows was restrain in lateral recumbency, secured with ropes. After aseptically preparation of the surgery and draping, an wedge resection involving the central third of the tumefaction was performed. The tissue was were resected by sharps dissections. The skin was apoosed by interrupted nonabsorbable suturesand a compresive bandage was applied. Postoperatively the cow received an meloxicam(Metacam) and antibiotherapy with ceftiofur(Cevaxel) for five days. The resected tissues were submitted for the hystopathologic exam and sample for microbiological and sensitivity test. The samples were stained using Hematotoxylin-eosin(H&E) and Masson's trichrome techniques.

Results

In this study the prevalence of tarsal cellulitis in the herd were 8 % , 19 cows were scored as having hocks, and 1,5% (n=4) had acropodial disorders. The progression of the disease into advanced phases could be controlled by adequate bedding, but

sometime the tarsal cellulitis lesions progress the ireversible phases. For this reason, in high genetics values cows the surgical ablation is an option in stopping worsening of the lessions, to prevent progression of the disease into advanced phases with infection infection and development of a chronic fistulous discharge of pus, severe pain and joint immobility, and endly drop milk production, and econimcal losses.The cows which underwent surgical ablation, tolerated well general anesthesia without any significant side effects as bloating, aspirating pneumonia. All cows recovered well after the surgery without septical complications or secondarylameness.Grossly, the tarsal region presented a focal nodular mass that was dense at palpation. In cross section, the tissue had a whitish to grayish appearance. Microscopically, there was a severe fibroplasia in the subcutaneous tissue represented by the irregular arrangement of the proliferated fibrous tissue. The suggested fibrous tissue was represented by thick collagen fibers and scarce fibrocytes and fibroblasts throughout the proliferated mass. In some microscopic areas the collagen fibers were concentrically arranged around blood vessels. The superficial epidermis undergoes acanthosis following moderate hypertrophy of the epidermic cells.

Conclusions

Traumatic and tumor-like disorders located at the lower legs can be highly prevalent in some dairy farms. Control of the lesions progression can be realized by proper bedding, appropiate mattress and bedded surfaces being associated with lower incidence and less severe hock injuries in this study. Surgical ablation of the pseudotumoral mass(tarsal and acropodial) represent an therapeutic option to prevent the further complications and to improve cows confort. In this study, the highest incidence of lower limb conditions associated with housing discomfort that could be surgically managed was tarsal cellulitis.

1632 - Thermography, could it be a tool to evaluate ringworm lesions in cattle?

Author: Ana Carolina Martins Bock, Rüdiger Daniel Ollhoff , Juliana Melo Pankratz, Diógenes Adriano Duarte Santana

Objectives

Investigate the possibility of early detection of ringworm lesions by thermal changes of the body surface caused by cattle trichophytosis.

Material and methods

During an initial ringworm outbreak in a herd of 71 Holstein cows, who were not vaccinated against trichophytosis (ringworm vaccine not available in Brazil), 46 of these cows began to show lesions resulting from ringworm. For the assessment, six cows were selected and randomly allocated into two groups. Group A (n=3) - cows without visible ringworm lesions. Group B exhibited an average of 6.66 lesions per cow, with a standard deviation of ±2.08. These lesions had an approximate diameter of 4.04 cm, with a standard deviation of ±1.76.

A Therma E50bx, (FLIR Systems, Sorocaba, Brazil) was used to measure surface thermal variations in both groups. The images were taken in a free stall without exposing the cows to direct sunlight. Therma E50bx, has a resolution of 240 x 180 Pixels equipped with a fixed 25-degree lens and thermal sensitivity <45mK. The average temperature (ºC) of the lesion was determined by calculating the mean of all pixels within the delineated area, while the minimum and maximum temperatures were automatically identified by locating the points with the lowest and highest temperatures. The exams were repeated once a week over a period of three weeks, also to verify if new lesions could be early detected.

Results

During the observed period, no cows in Group A developed lesions. In contrast, Group B cows maintained a consistent number of lesions, but the diameter of these lesions increased. Throughout the evaluations, Group A had an average skin surface temperature of 32.3 ± 2.83 ºC. In contrast, Group B had a mean skin surface temperature of 30.1 ± 1.79 ºC, and the temperature of the lesions in this group was an average of 30.4 with a standard deviation of 2.02 ºC. The thermography detected differences only when specifically directed towards the evident lesions in Group B. These lesions were already visible to the naked eye. When applying thermography comprehensively to the cattle, only lesions with a diameter greater than 4 cm were detected, and in Group A, no temperature change indicative of the onset of lesions was observed.

Conclusions

Although the thermograph is widely employed for various diagnoses, the assessment of cattle dermatitis such as caused by ringworm with the Therma E50bx was of no use. However, the possibility for an early thermographic detection system of skin inflammatory processes such as ringworm could in future be an interesting diagnostic tool to accelerate prophylactic and therapeutic measures in controlling infections, therefor contributing to better health and welfare in cattle.

1656 - Predicting the Treatment Outcome of Clinical Mastitis - Rationalising Antimicrobial Use

Author: Professor Andrew Bradley, Martin Green, Al Manning, Katharine Leach, Ian Glover

Objectives

The probability of cure of a case of clinical mastitis is associated with cow, pathogen and treatment factors. The aim of this study was to investigate the utility of a model, based on cow and herd characteristics available at the time of case detection, for predicting the probability of cure of a case of clinical mastitis during lactation and how this could be used to inform prudent use of antimicrobials.

Material and methods

Data for model training were collated from mastitis cases occurring on 52 UK dairy herds between 1994 and 2005. All herds undertook regular (monthly) DHI (Dairy Herd Improvement) testing and bacteriological diagnoses were available for a proportion of cases. A second dataset was built using 4,710 samples from 5 new herds using data collated between 2010 and 2020 to assess the ability of the models built to correctly predict treatment outcomes. Data were collated in TotalVet© which was also used to define treatment outcome based on non-recurrence of clinical disease and resolution to a low somatic cell count (SCC) (i.e. three cow level SCCs < 200,000 cells/ml or two <100,000 cells/ml) at routine DHI tests following the case of interest. Cow and herd level parameters of interest were then collated and analysed using Microsoft Access, Excel (Microsoft Corporation, Redmond, WA, U.S.) and R version 4.1.0. Cow/case-level and herd-level predictor variables were screened using univariable logistic regression. Associations between continuous predictors and the dependent variable were assessed using conditional probability density plots, and continuous predictors were categorised accordingly. Multivariable analysis was conducted using random intercept generalised linear mixed models (GLMM) with a logit link constructed using a forwards then backwards stepwise model selection.

Results

A total of 31,220 cases of clinical mastitis were available for analysis of which 32.8% were assessed as 'cured' using the definition outlined in the methods. A total of 22 case/cow level and 43 herd level variables were assessed. Independent variables in the final model consisted of features describing lactation stage, parity, previous somatic cell counts during current and previous lactations, previous history of clinical mastitis, position and number of affected quarters, season and herd average somatic cell count. Aetiology was not a useful predictor of treatment outcome once other herd/case characteristics were taken into account. Associations between independent variables and the probability of cure included age, somatic cell count history, the number of quarters affected and quarter location (fore vs hind) as well as the number of previous cases of clinical mastitis. Three examples of model predictions on cows with different characteristics in a herd with 25% of cows with a SCC > 200,000 cells/ml are: 1) a parity 2 cow, 150 days in milk (DIM) affected in a single fore-quarter experiencing her 1st case of clinical mastitis in her lifetime, with the last 3 SCCs below 30 x10^{3} cell/ml had a 65.5% cure probability; 2) a parity 6 cow, 140 DIM affected in a two hind-quarters experiencing her 2nd case of clinical mastitis in this lactation having experienced two clinical cases in

her last lactation with the last 3 SCCs greater than 2,000 x10^3 cell/ml had a 2.6% cure probability; 3) a parity 1 cow, 50 DIM affected in a single hind-quarter experiencing her 1st case of clinical mastitis in her lifetime, with the last 3 SCCs above 200 x10^3 cell/ml had a 23.6% cure probability.

Conclusions

The model developed as part of this research is predictive of the probability of cure of a case of clinical mastitis, and has undergone initial validation. The lack of influence of causal pathogen on 'cure probability' is worthy of note, though this perhaps reflects the fact that cow history (in particular SCCs) is likely to be predictive of aetiology rather than that causal pathogen is not influential *per se*. The model has the potential for use by farmers when making treatment decisions, based on the concept of "treatment worthiness". The results of this work are in accordance with other research which demonstrates that cow factors are associated with probability of cure of clinical mastitis.

1685 - USE OF AN ELECTROLYZED SUPEROXIDATION SOLUTION AS A PREVENTIVE IN THE PRESENTATION OF NEONATAL DIARRHEA IN HOLSTEIN CALVES

Author: Karina Loo Estrada

Objectives

To test the effect of an electrolyzed superoxidation solution (ESS) as a prophylactic tool for diarrhea in calves and its impact on antimicrobial use.

Material and methods

The trial started on November 24, 2022, at the Sociedad Productora Guadalupe S.P.R. de R.L. de C.V. dairy farm, which had 1200 cows in production and 150 calves from 0 to 70 days of age. Sixty female Holstein calves were assigned as they were born into two groups: 1) ESS group (n = 30), where the calves received colostrum or milk with 5 mL of electrolyzed superoxidation solution per liter. From the first colostrum feeding, continuing with each colostrum and milk feeding until 30 days of age; and 2) Control group (n = 30), where calves received colostrum or milk without any added product. For this trial, animals with low birth weight (below 37 kg), preterm, or from twin or dystocic birth were not used. Both groups followed the general management practices on the farm without any changes. Body weight and height were measured at birth, 30 days, and 60 days of age. Calves were monitored daily for any injury or disease problem. Rectal temperature, heart, and respiratory rates were measured when a calf presented diarrhea. If any of these measurements were abnormal, one of three treatment options was started. The first option was sulfamethoxazole with trimethoprim I.M. for three days. If the calf´s condition did not improve or presented diarrhea again two to three days after the first treatment ended, a second option was used, consisting of amikacin I.M. for three days. If the diarrhea continued or recurred after the second option, a third treatment option was used. This option consisted of a combination of kaolin and pectin, an antidiarrheal solution, and sulfamethoxazole, all administered parenterally.

Results

Calves of the ESS group had a higher average daily weight gain from day 30 to 60 of age than control group calves (0.767 vs 0.714 kg/day, respectively). Additionally, the ESS group calves were taller than the control group calves, gaining 1.6 centimeters more by 60 days of age. The use of ESS positively impacted the calves' health (X^2 =13.4, 0.05, df = 1; P = 0.0002), with only 57% of the ESS group calves and 96% of the control group calves being treated for diarrhea. Additionally, only 23% of the ESS group calves and 73% of the control group calves treated with option one for diarrhea needed the second treatment option (X^2 =11.9, 0.05, df = 1; P = 0.0005). There was no effect (X^2 =1.02, 0.05, df = 1; P = 0.3132) of ESS on the mortality rate, which was zero for the ESS group and 3.3% for the control group.

Conclusions

Adding electrolyzed superoxidation solution to the colostrum and milk during the first 30 days of life increased the growth rate of the calves. It reduced the percentage of calves presenting diarrhea, resulting in a decrease in the use of antimicrobials.

1690 - Prevalence of subclinical mastitis, economic losses and factors associated in dual-purpose bovine production units in the tropics

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Objectives

The objectives of the study were to determine the prevalence of subclinical mastitis, as well as the factors associated with its presence and economic losses that it may represent for dual-purpose cattle producers in the humid tropics of the state of Oaxaca.

In the Mexican tropics, there are more than 500 thousand cattle production units with a high percentage of producers with less than 30 cows. In the humid tropics of northern Oaxaca, the predominant bovine production system is dual-purpose, which allows the production of live cattle for sale or slaughter and milk production. However, production units in this region have low productivity, due to various factors such as diet and the presence of diseases. Among the most relevant health problems that affect dual-purpose cattle, mastitis is the main problem, this disease is caused by different etiological agents, generating considerable economic losses in dairy production.

Material and methods

6,232 functional quarters of 1,365 lactating cows from 12 herds were evaluated with the California mastitis test (CMT). The prevalence of subclinical mastitis was carried out in five ways, with the formulas used in previous studies: 1) prevalence in cows, 2) Prevalence by milking systems 3) prevalence in the total mammary quarters, 4) Prevalence in individual mammary quarters and 5) Prevalence in individual mammary quarters by position and degree of reaction in the CMT.

To determine the risk factors associated with the presence of subclinical mastitis, a format was stated with the producers help, and it was complemented by direct observation. The association of the prevalence and/or presence of subclinical mastitis with risk factors (management, infrastructure, animal and climate) was determined using Spearman's correlation coefficient. Economic losses were determined using an excel database, with necessary data, such as: the volume of milk production of cows that tested negative to CMT, the number of teats that tested positive to CMT of the 12 production units for each grade during the study period and the potential production volume. In addition, three scenarios of potential losses due to subclinical mastitis were determined taking as reference three different prices, as well as the variation in the percentage of reduction in milk production, according to the degree of subclinical mastitis of the teats that were positive.

Results

The prevalence of subclinical mastitis by cows, in the bovine herds analyzed was high, 41.7% (681/1,635) during the period studied, with November being the month with the highest prevalence.

In herds with automatic milking systems the prevalence is higher (47.7%), compared to the manual milking (34.7%); the prevalence by quarters was 23.7%. The most of the cows had grade 1 affectation in the quarters, followed by grade 2 affects (obvious

infection) and, finally, grade 3 (serious infection), which implies a decrease in the different degrees of affectation of the quarters.

There were factors associated with management, infrastructure, cow and climate, significantly associated ($p < 0.05$) with the presence of subclinical mastitis. The minimum potential economic losses were $144,399, and the maximum was $361,681 in Mexican pesos.

Conclusions

The prevalence of subclinical mastitis in dual-purpose cattle herds in the humid tropics in Oaxaca is high, with production units with mechanical milking with the highest prevalence in relation to those with manual milking. It is confirmed that subclinical mastitis is a disease that is associated with multiple factors (management, infrastructure, animal-related and climatic), and that the reduction in the amount of milk produced is a potential lost income of this livestock activity.

1713 - Evaluation of Dairy Cattle Locomotion, Eating, and Ruminating Behavior During Heat Stress Using Cutting-Edge Technological Monitoring

Author: Karina Džermeikaitė Džermeikaitė, Ramūnas Antanaitis, Justina Krištolaitytė

Objectives

Dairy farming is adversely affected by heat stress (HS). HS in dairy cows can result in decreased milk production, reduced reproductive rates, compromised immune function, and diminished animal well-being. Moreover, climate change is leading to an increased occurrence and severity of heatwaves, while the overall world surface temperature will continue to increase. High somatic cell count (SCC) is currently and will persist as a substantial concern for dairy farmers in the foreseeable future. Dairy farmers will face a persistent and major challenge of HS both currently and in the coming years. Non-invasive methods are predominantly employed to measure stress responses by analyzing alterations in behavioral and physiological reactions. Intelligent systems such as biosensors and innovative technologies, combined with sophisticated statistical models like machine learning and technologies like artificial intelligence (AI), can make a substantial contribution to achieving the intended objective. Multiple techniques exist for quantifying heat stress. One way to forecast heat stress is by utilizing the Temperature-Humidity Index (THI). Dairy cattle experience stress and a decline in their well-being and productivity when exposed to THI readings over 72. Dairy cows experience an inability to achieve their physiological requirements for milk production and overall well-being due to the reduction in energy intake caused by HS. The production and quality of milk are adversely affected, and the animals become more susceptible to disease. Based on heat stress monitoring data, the level of rumination in cows decreases as the THI increases. Elevated environmental temperature has a direct detrimental impact on the hypothalamic appetite center, leading to reduced consumption of food.

For these reasons, the objective of this investigation was to assess the impact of the Temperature-Humidity Index on rumination, feeding, and locomotor activity. The study evaluated rumination duration, drinking duration, number of gulps taken when drinking, number of chews per minute, number of chews per bolus, activity duration, and chewing duration during eating.

Material and methods

Analyzed were several parameters, including rumination time, drinking gulps, chews per minute, and others. The hypothesis posited that the implementation of precision dairy farming technology could facilitate the identification of HS. A total of nine Lithuanian Black-and-White cows, all in good health, were chosen at random for the trial. The RumiWatch noseband sensors documented behaviors, while the SmaXtec climate sensors monitored the THI. The data collection occurred between the dates of 14 June and 30 June.

Results

The hypothesis posited that the implementation of precision dairy farming technology could facilitate the detection of HS. Cows in the THI class with a value greater than or equal to 72 showed a significant decrease in drinking time (51.16% decrease, $p < 0.01$), a decrease of 12.9% in chews per minute ($p < 0.01$), and an increase of 16.99% in activity levels ($p < 0.01$). The THI exhibited a negative relationship with both drinking duration (r

= −0.191, p < 0.05) and chews per bolus (r = −0.172, p < 0.01). Advanced technologies such as RumiWatch are efficient in identifying the impact of HS on behaviors.

Conclusions

Dairy farming is significantly affected by the adverse consequences of HS. HS in dairy cows can lead to a decline in milk output, a fall in reproduction rates, and have a substantial impact on animal wellbeing. Non-invasive techniques are frequently employed to measure stress reactions by assessing alterations in behavioral and physiological responses. We have acknowledged that contemporary instruments such as RumiWatch, which incorporates a sensor in the noseband, can be employed to detect HS and its impact on the behaviors of rumination, feeding, and locomotion. In order to improve the welfare of cows, it is recommended that future research investigates the impact of heat stress on the biomarkers of RumiWatch sensors. This should take into account parameters such as the stage of lactation, the number of cows, milk production, and pregnancy status, while using a significant sample size. These findings highlight the potential of using novel methods to effectively manage heat stress and improve the overall well-being of the herd.

1714 - Improving udder health through a nutraceutical complement

Author: Frédérick Bussy NA, Preden De Cacqueray

Objectives

Mastitis is currently one of the most widespread troubles affecting dairy cattle with the highest economic impact. It has a significant negative impact on milk production leading to antibiotic use, high milk rules out …. Experimental evidence demonstrated that dry period mastitis can remain in a quiescent form and lead to clinical mastitis during subsequent lactation. To improve udder involution and maintain an udder well-being, several trials were performed with a plant-based product. The product named Stop Lactin gel is based on echinacea, parsley and sage, scientifically known as beneficial plants on udder health, B6 vitamins and chelated zinc to improve cells renewal.

Material and methods

Several trials were set up to evaluate udder congestion, milk leakage in early dry periods, colostrum quality, milk production and somatic cell count during the first month of lactation. Dairy cow farms were selected according to dry management (average dry period around 60 days). Only dairy cows with an average milk production exceeding 20 L the week before dry off, were included in the trials. The product (a complementary gel) was orally administered to one in two of these dairy cows (test group), the day before dry-off, after milking collection, and the following day at the dose of 70 ml.

Results

In the first farm, the somatic cell daily counts tend to be lower in the test group compared to the control group (respectively 47,107 and 38,612 cells per ml of milk). This result is in line with previous trials. This product helped to reduce mammary congestion and milk production, handling udder involution at dry off.

Conclusions

Previous trials, performed on sows, showed a reduction of udder engorgement, udder skin temperature and lower milk leakage during the days following weaning. Milk production was reduced quicker and with less inflammation in the test group. These results are consistent with the reduction of somatic cells during the early lactation period in dairy cows.

All in all, these results confirm that it can be possible to manage udder health during dry period thanks to a natural solution.

1732 - A novel production profile classification system for incoming calves that predicts feedlot growth performance

Author: Andreas Herman Reinhold Hentzen

Objectives
The aim of this study was to establish a criteria for optimal sorting of incoming feeder calves into various cattle groups in a feedlot that maximizes feedlot profit.
Material and methods
South African feeder calves (n = 436) were classified into four production profile (PP) categories according to a predetermined set of phenotypic traits: PP 3 (n = 72) representing feeder calves with the poorest feedlot growth potential, PP 2- (n = 191) with below-average potential, PP 2+ (n = 139) with above-average potential and PP 1 (n = 34) with above average feedlot growth potential. After combining the data of PP 2- and PP 2+ into PP 2, mixed modelling of economically important feedlot growth traits (average daily gain (ADG), carcass ADG, and carcass out weight) was performed to evaluate the effect of PP classification (PP 1 and PP 3) while adjusting for potential confounding effects such as starting weight (in-weight) and gender.
Results
: Carcass weight for calves with a PP classification of 3 and 1 were 15.54 kg less (P < 0.000), and 11.34 kg more (P = 0.007) than those with a PP classification of 2 (261.27 kg, 95% CI 257.94 – 264.57), respectively, after adjusting for in-weight, calf gender and the random effect of the feeding pen. Similar to carcass weight, calves with a PP 3 classification were outperformed by other classifications in all the measured traits (P < 0.05).
Conclusions
This is the first report demonstrating the ability of subjective production profile classification to predict growth performance of individual feeder calves before entering the feedlot phase. The future opportunity of the PP classification system lies in precision-feeding of calves based on their growth potential at the start of the feeding period, and then to use technology to improve and finalise the current subjective PP classification system.

1741 - Association between ATP luminometry of feeding equipment and environmental and health parameters of preweaned calves on dairy farms in Québec, Canada

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Objectives
The objective of this study was to examine the influence of different environmental factors on ATP luminometry measurements of feeding equipment and to investigate associations with health of preweaned calves and the levels of ATP identified through luminometry.
Material and methods
On 50 commercial dairy farms in Quebec (Canada), ATP luminometry measurements (in relative light units (RLU)) were obtained using the direct swabbing technique with Hygiena UltraSnap swabs and a liquid rinsing technique with the same swab for automatic milk feeders (AMF), bottles, buckets, esophageal tube feeders (ET), milk replacer, nipples and water. During this visit, environmental factors (including temperature, air draft, humidity, ammonia, and bacterial count) were collected and a clinical examination (including respiratory score and fecal score) was performed for all preweaned calves present at the farm. This process was repeated 4 times in a year, leading to collection of luminometer results, environmental parameters, and overall health of calves for each season per farm.
Results
Overall, a difference in luminometer results was seen between the different periods sampled for all feeding equipment (except the ET), milk replacer and water, showing higher RLU values in spring and summer and lower values in autumn and winter. When comparing RLU measurements with environmental factors, only a low to negligible correlation could be found. When feeding equipment was classified as not contaminated or contaminated based on previously described cut-off values, a good agreement within a farm for the different seasons was noticed only for nipples (Gwet's agreement AC1 = 0.64), with a poor to moderate agreement for other feeding equipment. Regarding the different models of nipples, 'Peach Teat' nipples showed higher RLU values compared to 'Merricks' nipples models. An association was seen between the proportion of preweaned calves suffering from diarrhea on the farm and the contamination of AMF based on ATP luminometry (logistic regression estimate = 0.52, P = 0.04). For other feeding equipment, milk replacer and water, no significant associations were found.
Conclusions
This study showed that ATP luminometry measurements of feeding equipment from preweaned calves are susceptible to seasonality and type of nipple. Thus, these factors should be taken into consideration when interpreting results. Additionally, an association could be made between diarrhea in preweaned calves and the contamination of AMF based on ATP luminometry, showing the potential clinical importance of this on-farm hygiene assessment tool.

1749 - On-farm rapid antimicrobial susceptibility test for decision-making on clinical mastitis treatment

Author: Breno Luis Garcia Garcia, Carlos Eduardo Fidelis, Gustavo Freu, Lígia Beatriz Rizzanti Pereira, Kristian da Silva Barbosa, Renata de Freitas Leite, Thatiane Mendes Mitsunaga, Diego Borin Nobrega, Marcos Veiga dos Santos

Objectives

Antimicrobial resistance (AMR) is a global concern which has been partially associated with the excessive use of antimicrobials in animal production. In dairy farms, the treatment of clinical mastitis (CM) is the main reason for the use of antimicrobials, and for this reason, the decrease in the use of antimicrobials for the treatment of CM may impact public health. Currently, the choice of antimicrobials to control CM is made without carrying out antimicrobial susceptibility tests, due to the time required to send samples and obtain laboratory test results. Considering that the rational use of antimicrobials in animal production has been associated with the reduction of AMR, the selection of antimicrobials for the treatment of CM based on rapid and direct in vitro antimicrobial susceptibility tests (RDAST) on the farm, can lead to a reduction in chances of AMR occurring in dairy herds. Therefore, the objective of the present study was to estimate the equivalence between the results of the RDAST methodologies and the standard agar disk diffusion antibiogram (ADD) methodology in CM bacterial isolates.

Material and methods

Bacterial isolates from CM cases of each of the following species were randomly selected: *Staphylococcus aureus* (n=70), *Streptococcus uberis* (n=48); *Klebsiella* spp. (n=57) and *Escherichia coli* (n=69) *Staphylococcus chromogenes* (n=70). The isolates were selected from the mastitis-causing bacteria library at Qualileite-FMVZ/USP, from 102 dairy herds. The isolates were previously identified by MALDI-TOF MS and stored at -80ºC. The susceptibility to antimicrobials of the isolates was evaluated using two methodologies: RDAST and ADD (standard methodology). The RDAST methodology was carried out by incubation in Muller Hinton Orientation chromogenic media (CHROMagar®, France), with direct inoculation of bacterial isolates from microbiological colonies. The standard methodology was carried out by inoculating standardized bacterial isolates at 0.5 McFarland (~10^8 CFU/mL), following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020). In both methodologies, the following antimicrobials were evaluated: amoxicillin+clavulanate, cephalexin, cephalexin/kanamycin, cefaperazone, cefquinome, ceftiofur, ciprofloxacin, cloxacillin, enrofloxacin, gentamicin, oxacillin, penicillin-novobiocin, pirlimycin. The inhibition halos in RDAST and ADD standard methodology media were categorized into three categories (sensible, intermediate and resistant) according to the inhibition zones (CLSI, 2020). The agreement results between the methodologies were calculated using Cohen's *Kappa* coefficient and the results were classified by level of agreement as: almost perfect (0.81 - 1.00); substantial (0.61 - 0.80); moderate (0.41 - 0.60); fair (between 0.21 and 0.40); slight (between 0.00 and 0.20), and poor (>0.00). Furthermore, Bland-Altman (BA) agreement analysis was performed between the inhibition zones of the isolates in each evaluation method, and the general agreement was measured by dividing the concordant results between the two methods by the total number of evaluations.

Results

Overall agreement between methods varied between pathogens from 0.712 (*Klebsiella* spp.) to 0.837 (*Escherichia coli*), while *kappa* results varied from 0.211 (*Streptococcus uberis*) to 0.446 (*Klebsiella* spp.). Considering the *kappa* interpretative categories, the RDAST showed moderate/regular agreement in relation to the standard method for all pathogens evaluated. The moderate/fair results observed may be associated with the lack of standardization of the inoculum, which can alter the diameter of the inhibition halo, causing errors in the interpretation of the results. BA results ranged from -4.872 mm (*Staphylococcus aureus*) to 1.532 mm (*Streptococcus uberis*) with a general bias of 1.946 mm, indicating that, in general, halos were larger in RDAST than in ADD.

Conclusions

The agreement between the RDAST methodology and the ADD was moderate, and an overall bias of -1.946 mm in the diameter of the inhibition halos was observed in the RDAST method. Interpretation of RDAST results must take into account the difference in the inhibition zones of each species, and further studies will be needed to evaluate RDAST as a method for selecting on-farm antimicrobials for CM treatment.

1766 - Optimizing Transition Health and Milk Performance in Holstein Dairy Cows through an Innovative Oral Effervescent Bolus Supplement with Live Yeast, Minerals, and Antioxidants

Author: RODRIGO GARCIA-LASTRA, MICHAEL REID, FIONNUALA McDERMOTT, ALFREDO SUAREZ-INCLAN, DAVID LOPEZ, ANTONIO FERRO , Maria Lourdes Auge

Objectives

Periparturient cows are confronted with challenges associated with parturition, environmental changes and drastic dietary changes which affect adequate dry matter intake (Goff, 2001). This study aimed to evaluate the effects of an oral bolus supplement containing live yeast, magnesium, calcium, potassium, and niacin on early lactation Holstein dairy cows.

Material and methods

From July to October 2023, on a commercial dairy located in Cordoba (Spain), 60 Holstein primiparous and multiparous cows were enrolled into one of two treatments over a period of approximately 100 days. Cows were blocked on parity, previous lactation milk production, fat and protein and randomized into two groups of 30. Cows assigned to the control group (CTL; n=30) received no oral supplement post freshening; the treatment group received one dose (220 grams) of the fresh cow bolus [YMCP Vitall®, TechMix, LLC., Stewart, USA] immediately following parturition (BOL; n=30). Daily and weekly milk production (kg) and health parameters were recorded (DairyPlan C21 management software, GEA, Germany). Results were statistically analyzed with SAS (version 9.4; SAS Institute Inc., Cary, NC) using a linear mixed model (PROC MIXED).

Results

Weekly average milk production was numerically higher from weeks 1 to 6 post calving for cows supplemented. Also accumulated milk production (kg) was higher for BOL relative to CTL cows at 30 DIM (days in milk) (+10.5%), 50 DIM (+7.6%), 60 DIM (+6.2%) and 100 DIM (+4.2%). BOL cows had lower incidence of retained placenta ($p<0.05$) and removals ($p<0.05$) during the first 30 DIM relative to CTL cows. Although no statistical differences were observed in milk production, clear numerical differences in the first 100 DIM in animals receiving the bolus are in line with previous studies (DeVries & Chevaux, 2014; Al-Qaisi et al., 2020).

Conclusions

Supplementing cows postpartum with BOL reduced the rate of retained placenta and removals during the first 30 DIM. These results suggest that supplementing cows postpartum with an oral bolus containing live yeast, minerals, and antioxidants is a valuable strategy to improve animal health and productivity in fresh cows.

1771 - Milk yield residuals and their link with behavioral patterns of dairy cows in the transition period

Author: Celien Kemel Kemel, Miel Hostens, Peter Hut , Matthieu Salamone

Objectives

The transition period between two lactations remains one of the most challenging periods during the productive life of dairy cows and is commonly known to be associated with diminished animal welfare and reduced economic performance in dairy herds. The development of data-driven health monitoring tools based on on-farm available milk yield data has shown potential in identifying health-perturbing events. Using data from the current lactation, comparing expected with realized milk yields makes monitoring the transition period difficult, due to the lack of milk data at the onset of lactation. For multiparous cows, an opportunity lies in the use of data from the previous lactation to predict the expected production of the next one. Recently, a random forest model (the NextMILK model) was developed, using milk yield data from the previous lactation to predict the first test day milk yield after calving. The expected milk yield was subtracted from the actual production to calculate the milk yield residuals in the transition period (MRT). Results of previous research suggest that MRT at the onset of a new lactation are indicative of the health and metabolic status of transition dairy cows. The main objective of this study was to link these MRT to different behavioral parameters, measured by two types of sensors of high-producing multiparous Holstein-Friesian dairy cows in the transition period.

Material and methods

Data from 2239 lactations of 1047 dairy cows on eight commercial farms in The Netherlands were used in the study. Lactations of primiparous cows were excluded due to a lack of historical milk yield data. Cows were fitted with two types of sensors, where feeding behavior (eating time and ruminating time) was monitored with the commercially available “Nedap Smarttag Neck” sensors (Nedap, Groenlo, The Netherlands) attached to the neck collar of each cow. The “Nedap Smarttag Leg” sensors were attached to one of the front legs of each cow, to monitor walking (walking time and standing time) and lying behavior (lying time).

The MRT were calculated using the previously mentioned NextMILK model based on test day milk records of the previous lactation, which were registered with an interval of 4-6 weeks as part of the dairy herd improvement program of the Dutch breeding organization CRV (Arnhem, The Netherlands). For further analysis, MRT were categorized into three quantiles (<33%; 33-66%; >66%).

Analysis of the data was carried out using R via the Google Colab system. Behavioral parameters were used as the dependent variable of interest. Linear mixed effect models were used for statistical analysis of each outcome variable. Initially, individual explanatory variables herd, parity of the animal, months in milk (-1 to 10), calving season, and MRT quantile were tested in univariable models. Akaike information criterion (AIC) values were used to select explanatory variables to build the multivariable model. Biologically relevant interactions of the explanatory variables with months in milk were included in the final model.

Results

Eating time for cows in the lowest MRT quantile was significantly lower during the dry cow period and the whole lactation, where they ate on average 6 minutes (95% CI: -8.6; -3.3) per day less than both other groups. For rumination time, no significant effects were found during the dry cow period and at the moment of calving. From the first month after calving till the end of lactation, rumination time for the cows in the lowest MRT group was significantly lower than for both other groups.

Lying time during the dry cow period was significantly higher for the best MRT group, where they lie on average 15 minutes longer per day (95% CI: 1.3-29.9). During the 2nd to the 8th month of lactation, the best MRT group had the lowest lying times. For standing time, during the dry cow period, the group with the highest MRT, had the lowest standing times, while during lactation (1st till 7th month), this group had the highest standing times. No significant effects were found for walking time within the different MRT groups.

Conclusions

The behavioral differences for cows with various MRT confirms the use of MRT as a metabolic indicator of transitioning dairy cows to predict the cow's adaptive capacity throughout the transition period and could support the development of new health monitoring tools, but further validation is needed.

1774 - Milk component ratios and their associations with energy balance indicators and serum calcium concentration in early-lactation spring-calving grazing dairy cows

Author: Ainhoa Valldecabres Inchaustegui, Pablo Pinedo, Ángel García-Muñoz, Louise Horan, Michael Dineen, Stacey J Hendriks, Juliette Masson

Objectives
Milk composition information from routine testing or new parlor technologies is becoming increasingly available in commercial dairy farms, growing the interest of its applicability under different production systems. Milk composition changes during energy deficient and low intake periods, such as that in early lactation. Thus, milk component ratios are expected to change during early lactation. Milk fat-to-protein ratio (FPR) has been commonly described as an indicator of energy balance and disease in confined production systems. Nevertheless, alternative milk component ratios (milk fat-to-lactose ratio (FLR) and milk protein-to-lactose ratio (PLR)) have been less commonly evaluated, and the association between milk component ratios and early lactation mineral balance has not been evaluated in either confined or grazing systems. The objectives of this study were to 1) describe milk component ratios from 0 to 45 days postpartum, 2) evaluate the association between milk component ratios (FPR, FLR and PLR) and indicators of energy balance (serum β-hydroxybutyrate (BHB) concentration between 5 to 45 days postpartum and body condition score (BCS) change during the transition period), and 3) evaluate the association between milk component ratios and serum calcium (Ca) concentration within 4 days postpartum in spring-calving dairy cows from 27 pasture-based commercial farms.
Material and methods
This observational study was conducted using information obtained from 548 cows housed in 27 spring-calving pasture-based commercial dairy farms in the Republic of Ireland. A total of 970 milk component results from samples obtained between 0 to 45 days postpartum, 918 serum BHB determinations obtained from 5 to 45 days postpartum, 851 BCS change observations during the transition period, and 50 serum Ca determinations within 4 days postpartum were used in this retrospective observational study. Multiple linear regression was used to describe milk component ratios and associations with the variables of interest.
Results
Overall, milk FPR, FLR and PLR were 0.70 ± 0.02, 0.53 ± 0.02 and 0.72 ± 0.01 during the study period (± standard error (SE)). Milk FPR linearly increased while milk FLR linearly decreased postpartum at a rate of 0.003 and 0.004 units per day, respectively; milk PLR decreased 0.02 units per day for the first 30 days postpartum and moderately increased afterwards. Milk FPR and FLR were 0.69 (SE = 0.02) and 0.52 (SE = 0.02) units lower before AM milking than before PM milking; while milk PLR was similar before AM and PM milking. Milk PLR was 0.02 (SE = 0.008) higher for ≥4th compared to 2nd - 3rd and 1st parity cows; while milk FPR and FLR were not affected by parity. Further, crossbred cows had 0.07 (SE = 0.03), 0.08 (SE = 0.02) and 0.03 (SE = 0.01) higher milk FPR, FLR and PLR than Holstein-Friesian cows. No associations were observed between milk component ratios and indicators of energy balance. A positive linear association was observed between milk FPR and serum Ca concentration within 4 days postpartum; milk

FPR increased 0.31 (SE = 0.14) units per each mmol/L increase in serum Ca concentration. Cows with low serum Ca concentration (<2.12 mmol/L) within 4 days postpartum had 0.27 (SE = 0.09) units lower milk FPR compared to cows above the threshold, and cows with low serum Ca concentration (<2.00 mmol/L) within 4 days postpartum tended to have 0.15 (SE = 0.08) units lower milk FPR compared to cows above the threshold.

Conclusions

In conclusion, milk component ratios determined before milking vary with milking time and may not be associated with energy balance indicators in early-lactation grazing dairy cows. The potential of milk FPR for monitoring blood calcium status warrants further investigation in early-lactation grazing dairy cows.

1776 - Effect of a lime-based bedding conditioner on physical-chemical characteristics and microbiological counts of recycled manure solids

Author: Gustavo Freu , Sara Fusar Poli, Valentina Monistero, Filippo Biscarini, Delower Hossain, Marcos Veiga dos Santos, Valerio Bronzo, Maria Filippa Addis, Paolo Moroni

Objectives
Bedding materials are aimed at providing a safe and comfortable resting environment for cows. Control of pathogen proliferation in these substrates is crucial to prevent intramammary infections in dairy cows. This is particularly relevant in the case of organic bedding substrates, including manure-derived materials. This study aimed to evaluate the effect of a lime-based conditioner **(LBC)** at various concentrations on the physical-chemical characteristics and bacterial counts of untreated anaerobically digested manure solids **(ADMS)** and separated raw manure solids **(SRMS)**.
Material and methods
Unused ADMS and SRMS were evaluated at four LBC concentrations: 0% (as untreated control), 10%, 15%, and 20% of LBC inclusion. The bedding materials were assessed immediately after LBC addition (0 h) and after 24, 72, and 168 h of storage at 28°C. The dry matter content **(DM)**, and pH were measured for all the time points. Standard microbiological methods were used to assess total bacterial counts **(TBC)**, other Gram-negative bacteria, coliforms, *Escherichia coli*, and streptococci and streptococci-like organism **(SSLO)**.
Results
It was observed a linear increase in both DM and pH with increasing concentrations of LBC. Specifically, for each percentage unit increase of LBC, the DM of ADMS and SRMS increased by 0.73% and 0.71%, respectively. Similarly, for each percentage unit of LBC, the pH of ADMS and SRMS increased by 0.15 and 0.19, respectively. Conversely, a linear decrease in TBC, Gram-negative bacteria, coliforms, *E. coli,* and SSLO was observed with increasing concentrations of the LBC. Manure-derived materials without the inclusion of the LBC had bacterial counts that tended to remain high or increase over time. Otherwise, bedding materials with LBC application had reduced bacterial counts.
Conclusions
Based on the results of the present study, it was observed that the higher the concentration of LBC, the more significant the reduction of bacterial counts. Specifically, bacterial recovery was lower when higher concentrations of LBC were applied. Our findings underscore the potential of LBC in effectively controlling environmental bacteria and optimizing the physical-chemical characteristics of manure-derived bedding materials to improve cow health and welfare.

1780 - Improving culling decisions based on trained artificial neural networks in Holstein dairy farms

Author: Oscar R. Espinoza-Sandoval, Einar Vargas Bello Pérez , Juan Carlos Angeles-Hernandez

Objectives
The objective of the present study was to train a model based on artificial neural networks (ANN) to predict the culling of cows in a Holstein dairy herd based on historical records.
Material and methods
To develop the model records from a dairy herd in northern Mexico (Chihuahua) with an average of 2000 dairy cows were obtained containing data such as milk production, days in milk, reproductive status, and lactation number. Also, records of dairy cows that were culling were obtained. The data was summited to the software R v4.2 and used the library *neuralnet*, to develop an ANN. The input data was normalized using the z-normalization before the ANN training. The architecture of the ANN was constituted of 2 hidden layers and with 9 neurons. Of the original data 80% was used to train the ANN and the rest 20% was used the test the prediction capabilities of the ANN. The mean square error was used to evaluate how far away the predictions were from the real data.
Results
The results after the ANN trained was tested shows a capability of 91% to predict the culling cows.
Conclusions
The present study shows the ability of ANN to perform complicated decisions on complex data with non-linear interactions like culling. Also, the current methodology proposed can be practical to implement in on-farm conditions.

1791 - Is vitamin D deficiency common in newborn calves in selected herds in Slovenia?

Author: Jože Starič, Rok Marzel , Lena Veren Geč, Jožica Ježek, Jana Avberšek, Špela Golavšek, Matjaž Ocepek, Branka Kramberger

Objectives

Vitamin D is an important nutrient with multiple functions in animal physiology, including bone development, immune function, and general health. Calves undergo rapid growth and skeletal development, especially in the early stages of life. An adequate supply of vitamin D is essential for the proper absorption and utilization of calcium and phosphorus, the most important components for bone mineralization. A positive correlation has been demonstrated between vitamin D supplementation and increased bone mineral content in calves, contributing to improved skeletal integrity and overall better growth. Vitamin D also has a modulating effect on the immune system. It has the potential to enhance the innate and adaptive immune responses. A well-functioning immune system is crucial for the prevention and control of infectious diseases, making vitamin D an important factor in calf health.

Material and methods

The study was conducted on 10 commercial dairy farms with different production systems. Forty-nine calves aged 1 to 7 days were examined for their blood vitamin D levels using immunoassay system (Mini Vidas analyzer, Biomerieux, France). We calculated the percentage of calves with vitamin D level above/below the threshold of 20 ng/mL indicating a deficiency.

Results

Our results show that 32 (65%) calves had a vitamin D blood level below 20 ng/mL and 14 (29%) even below 8.1 ng/mL, which is the lower limit of detection for the test used; 17 (35%) calves had a level above 20 ng/mL and of these, 10 (20%) had a level above 30 ng/mL (preferred level indicating adequate vitamin D supply).

Conclusions

In more than half of the calves tested, the vitamin D level was below the deficit value for calves. No calf reached the desired value in 8 out of 10 herds. Only in one herd was the value above 30 ng/mL (the desired value) in 8 out of 9 tested calves.

Vitamin D deficiency in calves is associated with increased susceptibility to infectious diseases and musculoskeletal disorders, so it is crucial to provide it to both dry cows, as they supply fetuses and newborn calves. Further studies are needed to investigate the importance of this issue, particularly in dairy herds, as most cows are kept indoors all year round, and to establish a standard for the vitamin D requirements of calves.

1792 - Veterinary consensus on the management of trichomonosis in South Africa to improve its control

Author: Dietmar Holm , Paul Reynolds

Objectives

Trichomonosis is endemic in South Africa, as is the case in most beef producing countries world-wide. Trichomonosis is not a state-controlled disease in South Africa and therefore control of the disease is the mandate of the private sector. A voluntary disease reporting scheme managed by the Ruminant Veterinary Association of South Africa (RuVASA) indicated that the number of herd outbreaks reported annually remains constant over the past decade. Anecdotal evidence indicates that private veterinary practitioners differ in their approach to the control of the disease, causing confusion in the industry. This questions the efficiency of current attempts to control the disease.

The objectives are to use trichomonosis as a model disease to establish consensus amongst private veterinary practitioners on diagnostic, reporting and control principles of non-state controlled diseases that significantly impact animal production. The ultimate goal is to decrease the negative impact of trichomonosis and other endemic diseases on animal production in South Africa.

Material and methods

Following a presentation and discussion at the annual RuVASA congress in 2023 (8 - 10 May 2023, Muldersdrift, South Africa), an electronic survey was conducted amongst RuVASA members to obtain their opinions about the need for consensus on trichomonosis control protocols, the standards required for sampling, laboratory testing and reporting of results, the standards required for certification of animals and herds as free from trichomonosis infection, and principles of controlling the disease in known infected herds.

Feedback was given at the 2024 RuVASA congress (hosted during the World Veterinary Congress, 15 - 19 April 2024, Cape Town), where an industry-wide best practie protocol for the testing, certification and control of trichomonosis in South Africa was adopted.

Results

Ninety-five (95) survey responses were received representing 34% of RuVASA membership, of which the majority (37%) were from the Free State, being the province in which trichomonosis outbreaks are reported most frequently. The majority (92%) of respondents were from practices that are involved with the testing and control of trichomonosis, and 23%, 38%, 22% and 17% of respondents declared that they handle <100, 100 - 500, 501 - 1000 and >1000 preputial samples annually, respectively.

The vast majority (92%) of respondents agreed on the need for an industry-wide approach, however of those involved in trichomonosis control, 39% were in support of a single protocol, vs 53% supporting differential protocols depending on the risk and/or specific circumstances on the farm. These opinions were not associated with the number of samples handled by the practice, nor with the province where samples are obtained from.

Equal proportions of respondents (17%) were of the opinion that the individual bull, vs the herd, should be certified free from trichomonosis, however the majority of respondents (62%) were of the opinion that it should depend on circumstances whether

certification should be done for the individual bull, the herd, or both. A small proportion of respondents (2%) were of the opinion that veterinarians should not certify disease free status for trichomonosis, but should just report their laboratory findings.
The majority of respondents (72%) were of the opinion that culture without PCR testing for trichomonosis should no longer be accepted, whereas 76% of respondents were in support of accepting pooled samples in a testing protocol as a means of making herd testing more accessible.
Respondents were split in their opinions whether treatment of bulls known to be infected with trichomonosis should be attempted or not(52% vs 48% respectively), and a tendency existed for respondents handling more samples annually to be more likely to be in support of treatment (62% vs 45%, $P = 0.13$). Those in support of treatment expressed strong views on this being a veterinarian-only procedure, a need for an evidence-based treatment protocol and emphasised the requirement to perform follow-up diagnostic tests several weeks after completion of treatment.
Discussions at the 2024 RuVASA congress aimed to resolve the outstanding matters in order to reach consensus.

Conclusions

It is concluded that the private veterinary sector in South Africa is ready to adopt industry-wide disease management protocols for non-state controlled diseases that significantly impact animal production. A disease management protocol for trichomonosis was adopted by RuVASA in 2024 with the purpose of reducing the impact of this economically important disease at a national level.

1803 - Impact of DIM at hyperketonemia diagnosis on milk yield, early reproductive performance, and herd removal in dairy cattle

Author: Juan Manuel Cainzos Cagiao, Jose Vilariño Quinteiro, Guillermo Lorenzo Diaz, Alfredo Suárez-Inclan Bernal, Ignacio Rico Adega

Objectives

High prevalence of hyperketonemia - defined as an elevated concentration of ketone bodies in blood, serum, or plasma (Doré et al., 2015) and traditionally considered an indicator of problems in the transition period (Rodriguez et al., 2022)– is still present in many Spanish herds (Guadagnini et al., 2019). Screening protocols to diagnose HYK typically involve the testing of cows during the first weeks of lactation (van der Drift et al., 2012). Previous studies have suggested that an increase in disease and culling events and a decrease in milk yield and reproductive efficiency are observed with lower BHB concentration thresholds in the first week (wk1) compared with the second (wk2; Walsh et al., 2007; Duffield et al., 2009; Rodriguez et al., 2022). The aim of the present study is to evaluate the effect of HYK evaluate the association of HYK in two different time point during early lactation (9 and 16 days in milk , DIM) with milk yield, reproductive performance, and herd removal throughout a single entire lactation.

Material and methods

The current retrospective cohort study was conducted on a commercial dairy farm in Carral (A Coruña – Spain). A convenient sample size of 441 cows was tested for hyperketonemia at both 9 and 16 DIM using a milk ketone test strip (Keto-Test; Elanco Animal Health, Greenfield, IN). Those animals testing with BHBA concentration of 100 µmol/L or above tested positive for HYK. Information about cow ID (CowID), date of birth (BirthDate), lactation group (LACT; 1,2 or ≥3), date of first AI (FstAIDate), date of pregnancy (IAFec), date of first test-day after calving (FstTestDate), first test-day milk yield (FstTMY, kg), data of culling (CullDate) and ketosis incidence at 9 (Ket9DIM) and 16 DIM (Ket16DIM, 0 or 1). Based on ketosis results at each test (HYK), cows were classified on either healthy (Healthy), positive at first test (1stT+), second test (2ndT+), or both (BothT+). Statistical analyses were carried out to evaluate HYK impact of these different days of testing on a) the incidence of the disease and b) on the consequences of a positive for ketosis in production (FrstTMY), reproduction (risk of pregnancy in the first 150 DEL) and culling (at 60 and 500 DEL). For the evaluation of the impact on milk production, multiple linear regression model was built for HYK values, including the lactation group and the DEL1stT as predictors. For reproductive and culling impact assessments, Cox regression analyzes were performed for Ketosis. Data have been managed through Gando 1.0 (Gando nuevas tecnologías SL, Spain) and all the statistical analyzes were carried out with STATA 14.2 (StataCorp LP, USA).

Results

20 out of 441 animals (4.54% incidence) tested positive to ketosis at 9 DIM, compared with 37 animals at day 16 (8.39% incidence). 389 animals were classified as healthy cows at both test (Healthy, 88.21%), 15 animals were positive only at 9 DIM (1stT, 3.4%), 32 at 16 DIM (2ndT, 7.26%) and only 5 were positive at both tests (BothT, 1.13%).

Compared with Healthy animals, 1stT+ yielded 7.94 kg less of milk at first test ($p<0.05$, $r^2=0.49$) but not for 2ndT+ and BothT+ animals (β=-2.22 and -5,14 and p=0.12 and 1.29, respectively). The 1stT+ animals had a 75% [hazard ratio (HR)= 0.25; 95%CI: 0.08 to 0.77]

lower risk of pregnancy within 150DIM and 3.36 times (95% CI: 1.53 to 7.38) higher risk of herd removal within 500 DIM than Healthy cows. Conversely, no differences between healthy cows and 2ndT+ for risk of pregnancy by 150 DIM (HR= 0.89; 95% CI: 0.51 to 1.56) or removal from the herd within 500 DIM (HR= 1.93; 95%CI: 0.43 to 8.59). For those cows Both+, only differences on risk for removal (HR= 5.62, 95%CI=1.75 to 18.07) were found compared with healthy animals.

Conclusions

Our results suggest that the DIM when ketosis diagnosed should be considered, as its association with performance outcomes might be different. Further research is warranted to understand the underlying causes of the effects of HYK at different time points and to investigate the use of a different threshold when diagnosing HYK at a different time in early lactation. All data used in these analyses were observational. So, only associations should be drawn from this study and further analysis are necessary for causality determinations.

1809 - IMPORTANCE OF BOVINE PARALYTIC RABIES IN THE MUNICIPALITY OF PIJIJIAPAN, CHIAPAS (CASE REPORT)

Author: Silvia Morales Méndez, Angel Yetzel Osio Martínez, Rubén Ramírez Navarro, Edgardo Canizal Jiménez, Daniel Morales Méndez

Objectives

The general objective of this work is to describe in detail a case of bovine paralytic rabies, placing special emphasis on the procedure to be followed once this disease is suspected, through the observation and follow-up of a case from its onset to the receipt of laboratory results and implementation of control and eradication measures. When comparing the official data of the cases reported in 2013 with the cases reported by a single veterinarian in the municipality of Pijijiapan, Chiapas, it was found that he is the only person who performs the complete diagnostic protocol, thus leaving a large proportion of cases undiagnosed.

Material and methods

This case was presented in a dual-purpose company in an extensive production system, with native grass, in the municipality of Pijijiapan, Chiapas. It is not supplemented with commercial concentrate, but mineral salts are supplied. The animal population is made up of two hundred cattle of zebu breeds and mixes of Brown Swiss American, of different ages. The immunization schedule consists of vaccines against respiratory complex and clostridial complex; the latter applied in the month of August. Previously, people had been vaccinated against rabies, but they had not been vaccinated for four years.

ANAMNESIS

On October 15 of this year, at the request of the producer, the production unit in question was attended to examine a male bovine of approximately 6 months of age, which presented incoordination and uncertain gait; The producer comments that from August 3 to date, three calves have died, which initially presented the same signs and subsequent prostration and sudden death. When he reached the paddock, he found the animal prostrate on its side; he was encouraged to get up and with a staggering gait, he collapsed again, from where he could no longer stand up.

GENERAL PHYSICAL EXAM

Among the main findings on physical examination were mydriasis, paralysis of the hind limbs, and a wound at the base of the ear resembling a bat bite. It is suggested to leave the animal in that place until it dies naturally to take the pertinent samples. We returned the next day to find out the animal's condition; it is in the same place. It is added to the clinical signs of ptyalism, neck flexed backward, and paralysis is generalized.

PRESUMPTIVE DIAGNOSIS

Bovine paralytic rabies

SAMPLING

After two days, the animal dies, and the head is taken to the Animal Pathology Center for brain extraction, according to the "Manual for Sampling: Bovine Spongiform Encephalopathy." Once the brain was removed, it was placed inside a resealable plastic bag and in a cooler with refrigerant for preservation. The SIVE-02 form for sample shipment was attached and sent to the CPA certified laboratory.

Results

As this is an endemic area of the disease, the rest of the herd was vaccinated to prevent future cases, even before receiving the laboratory result. Results are received electronically within approximately one week, confirming that the cause of death was bovine paralytic rabies.

Vaccination should be sufficient to protect all animals that have not been exposed to the rabies virus; on the other hand, those animals that show vampire bites are suspected of having the disease despite being vaccinated since the infection is already in process and the vaccine does not stop it. Therefore, it would not be uncommon for any other animal to die in a period of no more than two months.

Conclusions

According to the evaluations carried out in different production units of the municipality of Pijijiapan, Chiapas, a bias was found between the cases that are presented and those that are reported, largely due to the lack of information or knowledge of the farmer of the disease and his diagnostic protocol or his refusal to recognize the existence of the disease.

What is a reality is that in the municipality of Pijijiapan, rabies is a zoonosis of increasing importance, mainly due to the proximity of the population (both bovine and human) to the blood-sucking bat, this closeness maximized by the proliferation of palm groves that provide the bat with a perfect habitat, so it is important to describe cases to continue with the work of informing the population and contribute to the eradication of the disease from the municipality.

1812 - Monitoring the health of weaned dairy calves through voluntary weighing system

Author: Rodrigo Meneses Melo, Rafael Ferraz, Marcelo Ribas, Luigi Cavalcanti, Markus Araújo, Antônio Carvalho, Tiago Moreira, Elias Facury Filho

Objectives

The objective of this study was to evaluate the daily weigth gain of dairy calves using automatic scales as a monitoring tool for screening animals that might be diagnosed with tick fever (TF) and bovine respiratory disease (BRD). The objective of this study was to evaluate the daily weigth gain of dairy calves using automatic scales as a monitoring tool for screening animals that might be diagnosed with tick fever (TF) and bovine respiratory disease (BRD).

Material and methods

Fifty Girolando dairy heifers aged between 80 and 120 days were enrolled for 20 consecutive days. Automatic eletronic scales [Intergado®] were installed in front of water troughs, enabling the daily weighing when heifers climbed voluntary on the scale. Additionally, rectal temperature (RT), Winsconsin respiratory score, and blood smears were performed once a day, between 5:00 and 9:00 AM. Data on average daily gain (ADG) from the 10 days preceding (D-10) to the day of diagnosis (D0) of the first cases of each disease were selected, with the period D-10 to D-6 considered as the reference ADG for healthy heifers. A total of 202 RT measurements were taken, considering heifers with RT ≥39.4ºC as sick in the predictive model. ADG-related data were scaled to standarize results per animal. Feature engineering was employed to transform time series into 63 variables to discriminate between healthy and sick states. After discarding variables with high correlaction and low variance, 10 variables remained. A logistic regression was fitted to predict the sick or healthy state without considering the day of diagnosis. The model was evaluated using repeated 10-fold cross-validation for robustness. Sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated to assess performance. The identification of the hemoparasite in the blood smears and Wisconsin respiratory scores were considered the reference for TF and BRD diagnosis. Model performance was compared with RT measurement and ADG. Different scenarios were analyzed for the predictive model, varying false positive:false negative (FP:FN) value ratios from 1:1 to 1:5.

Results

Thirty-seven dairy heifers presented bovine babesiosis, primarily caused by *Babesia bovis*. The animals showed a decrease in ADG two days before and on the day of TPB and DRB diagnosis, respectively. Monitoring by body weight, using a 50% prediction cutoff from the statistical model, showed SE, SP, PPV, and NPV of 73%, 72.7%, 77.7%, and 67.5% for TF, and 67.3%, 80.2%, 66%, and 81.1% for BRD, respectively. Using a scenario where the value of a false positive (FP) case of TF is equal to false negative (FN) case, FP:FN of 1:1, the prediction cutoff of the model was 58.1%, revealing SE = 70%, SP = 81.8%, PPV = 83.3%, and NPV = 67.7%. For BRD, the FP:FN ratio of 1:1 generated a prediction cutoff of 52,4%, with SE= 65.4%, SP = 84.6%, PPV = 70.8%, and NPV = 81.1%. Generally, incresing the value of FN cases compared to FP cases for both

diseases increased SE and NPV while reduzing SP and PPV. RT obtained SE = 36.6% ; SP = 69.6%, PPV = 23.4%, and NPV = 81.2% for TF.

Conclusions

Monitoring through precise automatic scales was effective in the early detection of TF and BRD in sick heifers. Voluntary automatics wheighing demonstred superior screening capabilities in TF cases compared to RT mensurement. Therefore, the voluntary automatic weighing system is a promising technology for monitoring the health of calves in dairy production systems.

NUTRITION

1043 - Salmonella enterica serovar Dublin from cattle in California from 1993-2019: Ecological characterization and analysis of antimicrobial resistance

Author: Richard Pereira Pereira, Heather Fritz, Kristin Clothier, Kathy Toohey-Kuth

Objectives
The objectives of this study were to characterize AMR profiles based on clinical breakpoints for S. Dublin isolated from cattle in California from 1993-2019, evaluating ecological parameters for their composition and distribution. A second objective was to use MIC data for these isolates to identify antibiotics driving the evolutionary distinction over time. We hypothesize that we will identify changes in AMR diversity over time and that specific antibiotics will be identified as resulting in distinction in S. Dublin isolates when grouped in year groups.
Material and methods
This study used an ecological approach to evaluate antimicrobial resistance patterns in *S. Dublin* based on well-established clinical breakpoints, and variations in susceptibility as evaluated for multiple drugs at the same time using changes in antimicrobial susceptibility based on MIC data. Antimicrobial susceptibility data for *Salmonella enterica subsp. enterica serovar Dublin* (S. *Dublin*), a well-known cattle-adapted pathogen with current concerns for multidrug resistance, recovered from cattle at the California Animal Health and Food Safety Laboratory System (CAHFS) over the last 3 decades (1993 – 2019) was evaluated for antibiotic susceptibility. For this purpose, minimum inhibitory concentration (MIC) testing was conducted for 247 clinical S. Dublin isolates using Clinical Laboratory Standards Institute approaches (CLSI). Analysis of data included temporal changes in antimicrobial resistance (AMR) profiles, including by using ecological richness, diversity, and evenness approaches to compare samples from difference year groups (1993-99; 2000-05; 2006-10; 2011-15; 2016-19), age groups (early pre-weaned (PW); late PW; early heifer (HF); late HF and adult cow), geographic region (north, central, south), clinical signs (diarrhea vs. systemic illness), and season of isolation (winter, spring, summer, and fall). Linear discriminant analysis (LDA) was used to evaluate correlations between serial dilution MIC for each antibiotic and their correlation with year groups.
Results
Antimicrobial resistance richness, diversity, and similarity analysis revealed patterns for changes in AMR profiles for different age groups. Discriminant analysis using MIC log2 transformed data revealed changes in MIC for year groups, with a time-sequence pattern observed, with drivers for reduced susceptibility observed for 3rd generation cephalosporins and quinolones drugs observed for more recent year groups (2011-2015 and 2016-2019) when compared to older year groups (1993-1999 and 2000-2005).

Conclusions
Together, these results highlight the changes in the diversity of AMR profiles, as well as changes in susceptibility of S. Dublin over time for critical antimicrobials of importance to both animals and humans and support the need for continued monitoring and efforts that will support judicious use of antimicrobials, especially for these two drug classes.

1070 - Evaluating the potential of cultured species of Mexican tropical seaweed to reduce in vitro methane emissions in ruminants

Author: Erika Fernanda López Pastrana, Luis Corona Gochi, José Moisés Talamantes-Gómez, Daniel Robledo, Atmir Romero Pérez

Objectives

Reduction of enteric methane (CH_4) emissions from ruminants is of great importance because CH_4 is a potent greenhouse gas that contributes to global warming and it represents a loss of energy for ruminants. Previous studies have shown that some red seaweeds species supplemented in the diet of ruminants can drastically reduce enteric CH_4 emissions. Thus, the main objective of this study was to evaluate three Mexican cultured red seaweed species from the Yucatan Peninsula to determine their potential to inhibit metanogenesis and its effects on rumen fermentation *in vitro*

Material and methods

The experiment was arranged as a completely randomized block design with a 3 × 2 factorial arrangement of treatments, including 3 seaweed species (*Rhodymenia pseudopalmata*, *Gracilaria cornea* y *Solieria filiformis*) and two doses (10 and 20% of dry matter). A control without seaweed was included in parallel. Three independent batch culture runs were conducted using alfalfa as substrate. A total of 1 g of alfalfa and seaweed was weighed into nylon filter bags and deposited in amber glass bottles. The rumen liquid used as inoculum was obtained from four sheep after the morning feeding. Bottles were filled with a combination of inoculum and buffer solution and incubated in a water bath for 72 h at 39°C. Total gas production (TGP), CH_4 production, gas production kinetics, digestibility of dry matter (DMD), and volatile fatty acid concentration (VFA) were evaluated. Data were analyzed with the MIXED procedure of SAS using the Tukey instruction for mean comparison.

Results

The effects of specie ($P = 0.56$) and the specie × dose interaction were not significant ($P = 0.44$) for DMD (g/100 g DM). However, DMD decreased ($P < 0.05$) up to 6.96% when the inclusion dose was 20%. Consequently, a reduction (10.40%) in TGP (mL) was also observed for the highest dose of seaweeds ($P < 0.01$) with no significant effects for specie ($P = 0.36$) or the interaction specie × dose ($P = 0.69$). The concentration of total VFA decreased ($P < 0.01$) by 7.5% with the inclusion of 20% seaweeds; however, the concentration of acetate and propionate was not affected by treatments ($P \geq 0.89$). As expected, the reduction in total VFA was associated with the increase in rumen pH observed when seaweeds were included at a 20% concentration ($P < 0.01$). The interaction specie × dose for CH_4 production (mL/g DM) was significant ($P = 0.03$) and *Gracilaria cornea*, *Rhodymenia pseudopalmata* and *Solieria filiformis* reduced emissions by 31.5, 24.6 and 33.5% respectively when included at 20%. Moreover, the inclusion of 10% of *Rhodymenia pseudopalmata* and *Solieria filiformis* reduced emissions by 15.7% when compared to control. The reduction in CH_4 production was partially explained by the reduction in DMD; however, it also resulted from a significant reduction ($P < 0.01$) in the concentration of CH_4 in the produced gas.

Conclusions

All the seaweed species evaluated decreased methane emissions at the two doses evaluated, except for *Gracilaria cornea* included at 10% of DM. Although a slight

reduction in digestibility was observed when different seaweed species were included at 20% inclusion, an average reduction in methane production of nearly 30% was also observed with a small impact on fermentation variables. In conclusion, the use of tropical red seaweeds evaluated in the present study, might be an option to reduce methane emissions in ruminants; however, our results must be further verified with *in vivo* experiments.

1082 - Effect of injectable or oral trace mineral supplementation on beef calf health status

Author: Nicolas HERMAN, Luc DUREL , Sébastien GEOLLOT, Agnès BATARD, Thibault DEVAMBEZ

Objectives

In Auvergne, a mountainous region in central France, geological conditions expose cow-calf herds to marginal deficiencies in Iodine (I) and Selenium (Se), to name just these elements. Depending on their intensity, deficiencies can occasionally predispose cows and calves to various disorders. To prevent the occurrence of the most severe troubles, cattlemen distribute multi-mineral and vitamin supplements to pregnant cows and a dietary supplement in the form of a solid bolus of Se to newborn calves, despite the lack of manufacturer's recommendations. This study aimed to compare the relative effects of the dietary supplement and an injectable multi-mineral medication on the health and growth of calves during the first six months of life.

Material and methods

Researchers used the selection of twelve calf-cow farms under the regular supervision of the local herd improvement organization (Cattle Croissance) to further this study. They made it possible to bring together a study population of 600 cow-calf pairs. Researchers supplemented pregnant cows with a vitamin and mineral complex at the end of the grazing season (November). Mineral status of cows was checked twice, before supplementation and at calving. At birth (D0), researchers identified the calves and weighed them. Researchers gave the cow calves a tablet (OTM group) containing 20 mg of Se as sodium selenite (OROSEL®, Octavet, France) (n=300) or injected (ITM group) with 1 mL/50 kg BW, SC, of a multi-mineral solution (MULTIMIN®, Virbac, France) containing Cu (15mg) Zn (60mg), Mn (10mg) and Se (5mg). Researchers repeated the injections at D30 and D60 and weighed the calves at four and seven months old. Colostrum quality was assessed using a Brix refractometer (°Bx) on the secretion collected as soon as possible after calving. Mortality and the incidence rate of diseases, including diarrhea, omphalitis, pneumonia, and application of medicinal treatments, were recorded from D0 to D210.

Results

The cows' plasma concentrations were standard for Zn and Cu. By contrast, they were low regarding inorganic I and Se in almost 33% and 41% of test animals, respectively. In both treatment groups, most calves experienced a successful passive transfer of immunity. The logistic regression in the population showed that the incidence rate of diarrhea, death, and antibiotic treatment during the study was strongly negatively correlated with °Bx ($P<0.01$, all endpoints). Omphalitis affected 14.2% (85/600)of the calf population. The odds of developing navel infection were lower, at 11.0% in the ITM group, compared to the OTM's 17.3%, a significant contrast ($P=0.035$). The cumulative incidence rate of all health troubles was significantly lower in the ITM group than in the OTM group ($P=0.007$). Except for pneumonia (12.3% vs. 13%, for the OTM and ITM group, respectively), the incidence of diarrhea (24% vs. 22%), use of oral (7% vs. 6%) or IV rehydration therapy (4% vs. 2%) or use of antibiotics (43.3% vs. 38.0%) and mortality (3% vs. 2%) were numerically higher in OTM group than in ITM group (n.s.). Researchers reported Various diseases such as arthritis, coccidiosis, undifferentiated fever,

meningitis, septicemia, tracheitis, and interdigital phlegmon. ITM animals showed significantly lower odds of getting other conditions than the OTM animals (n=6 vs.16, OR=0.36, CI95%[0.14;0.94], P=0.037).

Conclusions

Nowadays, oral se supplementation is the primary way of supplementing newborn calves in cow-calf herds in France. However, data on supplementing newborn and pre-weaned beef calves are scarce. In this study, ITM supplementation was as efficient as oral supplementation regarding the health status of the calves. But for pneumonia, calves in the ITM group experienced fewer health problems overall during the study period. These findings accentuate the importance of the supplementation strategy and colostrum quality in enhancing calf health and reducing the incidence of specific diseases, particularly omphalitis and overall morbidity.

1102 - Effect of a pre-calving injectable trace mineral supplement on white blood cell function in seasonally calving pastoral dairy cows

Author: Andrew BATES, Matt WELLS, Clare FITZPATRICK, Richard LAVEN

Objectives
To investigate the effect of a SC injection 14–28 days before calving, of a trace mineral supplement (TMS) containing 200 mg zinc, 50 mg manganese, 25 mg selenium, 75 mg copper on white blood cell population, function, serum antioxidant capacity (SAC) and reactive oxygen species (ROS) after calving.
Material and methods
On two convenience-selected seasonal calving, pastoral dairy farms, a random sample of 150 multiparous cows calving within ±7 days of the herd's planned start of calving (PSC) were identified one month preceding dry off. Cows were stratified on individual somatic cell count, age, breed and expected calving date. On each farm, 14–24 days before PSC, 60 of the selected cows were randomly assigned for TMS and 60 as controls. All 240 cows were contemporaneously injected S/C with 12,000 µg hydroxocobalamin, with controls also receiving 24mg selenium. On each farm, blood samples were collected at enrollment and from 100 enrolled cows at 4±4, 14±4 and 40±4 days after calving and the phagocytic activity, proportion of neutrophils, lymphocyte and monocytes, ROS, SAC and their ratio (ROS/SAC) measured. Concurrently, serum concentrations of Se and Cu and whole blood glutathione peroxidase (GPx) were monitored from a random subset of animals.
Results
Over the follow-up period, complete datasets were available from 171 cows for analysis of white blood cell parameters and from 52 for Cu, Se, and GPx. Multivariable, hierarchical Bayesian analysis indicated differences from TMS adjusted for farms and time. Over the entire period of follow-up, in cows receiving TMS pre-calving, 2,900 more bacteria were ingested/cell (95% probability between 2,600–3,200 bacteria/cell). Although the proportion of white blood cell types remained within normal limits, there were differences in the white blood cell population over the period of the study, between cows receiving TMS and control cows. In cows receiving TMS pre-calving, at 3 (range 0–9) days after calving, there were 7% more white blood cells expressing phagocytosis (95% probability between +2–12%) but 6% fewer neutrophils (90% probability between −11 and 0%). At 12 (range 8–18) days after calving, in cows receiving TMS pre-calving, there were 10% more lymphocytes/mL blood (95% probability between +3.0–18.0%) and at 40 (range, 36–45) days, there were 5% more lymphocytes (95% probability between +9–0%) and 4% fewer neutrophils (90% probability between 8–0%). There were no meaningful biologically important differences over the study period in ROS, SAC, their ratio, the proportion of monocytes, or the absolute number of white blood cells. Serum Cu and Se and plasma GPx concentrations were above the recommended threshold for all samples, although the serum Cu was 1 (95% probability interval = [−0.5;2.5]) µmol/L higher in cows receiving TMS.
Conclusions
Pre-calving injection with TMS was associated with differences in white blood cell population and function. These effects may counter the periparturient decline in immune function over calving and reduce the risk of clinical disease.

1103 - Injectable supplementation of trace minerals alleviates summer milk fat depression and improves reproductive performances in grazing dairy cows in France

Author: Olivier SALAT, Luc DUREL , Sébastien GEOLLOT, Guillaume LEMAIRE, Florent PERROT, Denis Jarrin

Objectives

Milk production is a significant metabolic challenge for dairy cows, requiring high energy input. This challenge is even more pronounced for cows that switch between a winter diet and summer grazing, affecting the evolution of milk solids concentration, particularly milk fat. When cows graze, the concentration of milk fat may drop, leading to milk fat depression. This study aims to compare the evolution of milk fat concentration during winter and summer in two groups of cows, one of which received an injectable mineral supplement. Moreover, the study also examines the supplement's impact on the time to conception.

Material and methods

Researchers followed 355 dairy cows from 12 farms in France for 18 months, from 2021 to 2022. The cohort included 36% primiparous cows. Enlisted cows were divided into the MM group (n=177) and the C group (n=178). Cows in the MM group received an injection of 1 mL/100kg SC of Multimin™ Injectable Solution for Cattle (Virbac, France), a multimineral solution (60 mg/mL Zn, 15 mg/mL Cu, 10 mg/mL Mg, 5 mg/mL Se). Animals were first injected 60 days before the expected calving date, followed by a second injection 30 days later and the third injection at 35 days in milk. The cows in the C group did not receive any treatment. Milk fat (MF) was measured four times (M1, M2, M3, and M4) once a month, starting from the first check shortly after calving, according to the official French dairy herd improvement organization (France Conseil Elevage) guidelines. Time from calving to first artificial insemination (AI) or conception was calculated from pregnancy checks and insemination dates. The milk fat analysis was performed on 50 animals in winter and 100 animals in summer for each group. The LOCF (Last Observation Carry Forward) method was applied when data was missing. For the analysis of time to conception, each group included 35 animals for each season. The summer season is defined by having a day length of more than 10h40mn at the average latitude of the selected farms (45°N).

Results

The evolution of MF showed a reduction between the first and second control before recovering or increasing in the third and fourth control. This pattern varies depending on the group of animals and the season. During the summer season, the average MF value between M1 and M2 had a difference of -0.67±2.48 and -1.70±2.53 g/L (n.s.) for groups MM and C, respectively. During winter, the reduction was -1.34±2.39 and -3.06±2.62 g/L (n.s.). When comparing the evolution of MF on the four controls, we observed a significant difference between the winter season (high MF) and the summer season (low MF) for animals in group C (P= 0.026). In contrast, the MM group has no seasonal difference (P=0.919), even if MF is slightly higher in winter than in summer.

The MM group had a shorter time between calving and the first AI than the C group (73.8 vs 78.6 days, n.s.). However, the time between calving and the first AI was significantly longer (p=0.035) in summer than in winter for animals in group C (+12.8

days). The average time to conception was considerably shorter (P=0.037) in the MM group, with 78.8 and 85.7 days for the MM and C groups, respectively. The same trend was observed in summer, with 78.3 and 86.6 days (P=0.056) for groups MM and C, respectively. In winter, the trend continued with 79.7 and 84.0 days (n.s.) for the same groups.

Conclusions

Results from this study conducted on dairy cows that were subject to seasonal grazing suggest that dosing animals three times systematically with trace elements (copper, zinc, manganese, and selenium) has the potential to decrease milk fat depression syndrome, and improve specific reproductive parameters, including the interval between calving and first insemination, as well as the time between calving and conception. Further research is needed to connect these findings.

1136 - Effect of two recombinant bovine somatotropin products on milk production and feeding parameters of lactating Holstein cows

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Objectives

This study evaluated the effect of two commercial products of recombinant bovine somatotropin (rbST) on milk production and feeding parameters in lactating Holstein cows.

Material and methods

The study was conducted at a commercial dairy farm in the southern region of Brazil. Eighteen cows were randomly divided into two groups, i.e., "rbST-Slow" and "rbST-Fast" and treated respectively with two rbST formulations: Lactotropin® (Agener União Saúde Animal, SP, Brazil) and Boostin® (MSD Saúde Animal, SP, Brazil). Both products were administered subcutaneously in mid-lactation Holstein cows, in five applications at a 14-day interval. Milk yields were measured daily using the DelPro™ software (DeLaval®). Feed consumption, feeding behavior and day-to-day variability in feed intake were also assessed daily using automatic individual feeders (Intergado®, SP, Brazil). Body weight and body condition score (BCS) assessments were performed on the day of each treatment application. Blood samples were collected on days 1, 4, and 7 post-treatments of each administration cycle, to assess circulating levels of non-esterified fatty acids (NEFA).

Results

Cows in the "rbST-Fast" group produced significantly more milk as compared to the "rbST-Slow" group (P=0.03). The" rbST-Fast" group tended to present greater feed intake (P=0.07), but no impact was noted on day-to-day variability of feed intake (P=0.64). In addition, animals in the "rbST-Fast" group exhibited more frequent visits to feeders, longer time in consumption, and higher relative Dry Matter Intake (DMI) per percentage of live weight (P<0.01). Furthermore, the mean weight of the cows was significantly different (P=0.05) between groups, but no difference was noted in BCS (P=0.30) between the groups. The animals in the "rbST-Fast" group had significantly higher concentrations of NEFA than those in the "rbST-Slow" group (P<0.01).

Conclusions

Administration of "rbST-Fast (Boostin®) resulted in significant higher milk yield compared to the "rbST-Slow (Lactotropin®)" group. This increased milk yield in Boostin® group was not accompanied by the day-to-day variability in feed intake.

1139 - Effect of inclusion of aluminum silicate in finishing diets for lambs: Growth performance, dietary energy, and carcass characteristics

Author: Jesús David Urías Estrada, Daniel Alejandro Mendoza Cortez, Beatriz Isabel Castro Pérez, Elizama Ponce Barraza, Yesica Janeth Arteaga Wences, Jorge Luis Ramos Méndez , Alejandro Plascencia Jorquera, Alfredo Estrada Angulo, Lucia de Guadalupe Escobedo Gallegos

Objectives

The use of clays as a feed additive in animal diets has been gaining greater interest in recent years. Among the most used clays are those from the aluminosilicate family. [Azomite® (AZ)] is characterized by containing up to 5 times more mineral elements than other members of the aluminosilicate family (i.e. zeolites) commonly used in animal feed. The greater content in mineral profile for AZ could represent an advantage for animal productivity. In this sense, inclusions of 0.25 to 1.0% of AZ in diets for broilers have improved weight gain and/or feed conversion (Ahamed et al., 2019). However, to our knowledge, there is no information on the impact of the use of AZ in finishing diets for ruminants. For this reason, the objective of this study was to evaluate the effects of AZ inclusion in finishing diets for feedlot lambs on growth-performance, dietary energy utilization and carcass characteristics.

Material and methods

Thirty six Pelibuey × Katahdin crossbred intact male lambs (18.81 ± 3.04 kg) were used in order to evaluate the effects of AZ inclusion in finishing diets on growth-performance and carcass characteristics. Lambs were grouped by initial weight and assigned within six weight groupings to 18 pens (2 lambs/pen, 6 replicas per treatment) in a randomized complete block design (criterion of blocking= initial weight). Diets offered (twice daily) were corn-based finishing diets with a 88:12 concentrate-to forage ratio. Treatments were as follows: 1) no clay inclusion (CTRL), 2) AZ inclusion at 0.75% level (0.75AZ) in diet DM, 3) AZ inclusion at 1.50% level (1.5AZ) in diet DM. Feeding trial lasted 81 d. Dry matter intake and changes in body weight was registered during the experiment. Dietary energy utilization was estimated based on performance data according to Estrada-Angulo et al. (2021). After the feeding trial was finished, all lambs were sacrificed following humanitarian procedures and carcass characteristics and tissue composition of shoulder were evaluated. The data were analyzed as a randomized complete block design, with the pen as the experimental unit. Treatment means were separated using the “honestly significant difference test” (Tukey’s HSD test). In all cases, the least squares mean and standard error are reported, and contrasts are considered significant when the *p* value <0.05.

Results

As a result of inclusion of AZ into diets, crude protein and net energy were slightly lower in AZ diets. There were no differences between treatments on water intake and average daily gain, but numerically differences in DMI for lambs that were fed with diet contained 0.75AZ results in improves (p <0.05) of 4.5% in gain efficiency. The efficiency of utilization of dietary energy (observed-to-expected dietary energy) was close 1.00 in CTRL and 1.5AZ groups (1.00 and 1.02, respectively), while in 0.75AZ group, the efficiency of energy utilization was increased by 5%. Dressing percentage was greater for lambs receiving the AZ treatments, while *Longissimus* (LM) area were greater to

1.5AZ treatment, intermediate to 0.75AZ and lower to CTRL treatment. Kidney-pelvic-heart fat was greater to CTRL, intermediate to 1.5AZ, and lower to 0.75AZ treatment. Compared to CTRL, muscle tissue in the shoulder was greater in lambs that received the AZ diets. However, the muscle to fat ratio only was significantly different when compared CTRL vs 0.75AZ group.

Conclusions

It was concluded that AZ can be included as a feed additive in finishing diets for lambs. Compared to non-supplemented lambs, the inclusion of 0.75% in diet showed better response in feed efficiency, dietary energy utilization, and dressing percentage; while the inclusion of 1.50% AZ in diet only improved dressing percentage and LM area.

1267 - Risk factors for insufficient silage quality in dairy farms in three different regions in Germany

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Objectives

Conserved feed stuff such as silages are widely used as major components in modern dairy cow diets. Quite often silages do not meet the quality requirements which negatively affect palatability and feed intake and may cause severe disturbances of ruminal function and even intoxication with far-reaching negative impact on animal health. It was the aim of this study to identify risk factors for insufficient fermentation and microbiological quality of grass (G) and corn (C) silages obtained from dairy farms in three structurally different regions in Germany with intense dairy cow farming.

Material and methods

As part of a large cross-sectional study performed in Germany, data were collected on 666 dairy farms in the region North (N) (n=237), region East (E) (n=227) and region South (n=202), which stored their silages in bunker silos. During an one-time visit, samples of silages from open silos which were actually fed to the dairy cows were collected and the fermentation and microbiological quality was assessed. Furthermore, information of the silage management was obtained via an interview with the farmer. Fermentation quality was evaluated based on the content of butyric acid, acetic acid, dry matter (DM) and pH. With respect to fermentation quality, silages were categorized in acceptable (very good to good fermentation quality; 72-100 points) and deficient (mediocre to poor fermentation quality; 0-71 points). With respect to microbiological quality, silages were categorized in acceptable (score 1), slightly deficient (score 2), severely deficient (score 3) and spoiled (score 4). For statistical analysis, score 1 (acceptable) was compared with the combined scores 2-4 (deficient). Hyopthesis driven and following association and correlation studies, possible risk factors were identified and tested in univariable and multivariable mixed general linear models for their effect on fermentation and microbiological quality.

Results

Overall, 1,475 silage samples were collected (n=843 grass silages (G), n=632 corn silages (C)). Almost all C had good fermentation quality (overall: 98.1%; N 97.8%, E 100.0%, S 96.1%), whereas in G, only 68.1% had good fermentation quality (N 72.5%, E 75.1%, S 55.3%). Acceptable microbiological quality was found in 58.1% of C (N 46.9%, E 73.5%, S 52.8%) and 75.6% of G (N 68.2%, E 79.4%, S 82.3%). For G, a crude ash content $\geq$10% DM was found to be a risk factor for low fermentation quality in N (OR= 2.8) and E (OR=2.6). Other risk factors were the lack of silage additives (N OR=3.5) and, unexpectedly, an even cutting surface (S OR=2.5). For C, no multivariable models could be built due to the lack of variation of the target variable. Univariable analysis did not reveal any risk factors for N and E. For region S, a crude ash content of $\geq$4.5% DM (OR 7.1) and a silo without side walls (OR 15.9) turned out to be risk factors for low fermentation quality. With respect to microbiological quality, an ensiling time of < 6 wks was identified as risk factor for G (N OR=3.9, S OR=5.9). For C, the model did not reveal

risk factors in N, whereas in E, a short ensiling time of < 6 wks and the lack of side walls of the silo were risk factors (OR 3.6 and 3.2, respectively). For region S, the year of the visit (2018 vs. 2019) turned out to be a risk factor for deficient microbiological quality of C (OR 2.7).

Conclusions

Silage quality based on fermentation and microbiological characteristics differed between region and was better in region East than in region North or South. Whereas fermentation quality was better in C than in G, the microbiological quality was more often deficient in C than in G. Some of the investigated risk factors repeatedly turned out to effect silage quality negatively: a high crude ash content, lack of silage additives and silo without side walls with respect to fermentation quality, and short ensiling time and silo without side walls with respect to microbiological quality. Our results suggest that an improvement of harvesting techniques, ensiling management and silo construction might help to produce silages of good quality.

1283 - Evaluation of the supply of xylanases and Aspergillus oryzae spores on performance parameters of growing beef steers as an alternative to monensin

Author: Pedro Martin Sueldo , Gonzalo Rodrigez Senes

Objectives

Feed additives are included into finishing diets to improve cattle growth and feed efficiency, resulting in enhanced productivity and profitability in feedlot systems (Callaway et al., 2003; Vohra et al., 2016).

Consumer concern about antibiotic use in animal feed, combined with the need for a safe food supply, has renewed research and development of natural feed additives. One option is the addition of enzymes and probiotics to animal diets.

The objective of the present study was to evaluate the effect of supplying a commercial product based on xylanases (500 UI/g of activity) and Aspergillus oryzae spores ($2x10^4$ UFC/g) on the performance parameters of finishing beef steers as an alternative to ionophore monensin.

Material and methods

In the present study, all animal handling procedures followed the guidelines of the Institutional Committee of use and care of experimental animals of the National Institute of Agronomic Technologies (INTA) – Republic of Argentina.

This experiment was conducted at the Research and Development Center CONECAR, located in the town of Carcaraña, Santa Fe, Argentina. One hundred and eighty Braford steers were housed in a common feeding facility 21 days prior to the beginning of the experimental period (day 0) for acclimation to pens and feed bunks. One transitioning total-mixed ration (TMR) were offered to steers prior to the beginning of the experiment and all included monensin.

On day 0, steers were ranked by full initial body weight (BW) (315 ± 30 kg), and randomly assigned to receive the finishing TMR containing their respective treatments. Steers were allocated to 1 of 12 pens (8 × 23 m) in a manner that average BW was equivalent across pens. There were 15 steers per pen and 6 pen per treatment (pen=repetition). Steers were confined for ninety-three days.

Control (C) diets included 30 ppm of the ionophore monensin (Rumensin™ 90 Elanco Greenfield, IN, US). The experimental (EX) diet included 0,2% DM basis of the enzyme-probiotic additive without monensin. The diet contained (on DM basis) 49.1% ground corn grain, 27% dried distillers grain with solubles, 11.3% wheat bran pellet, 10.2% peanut hulls and 2.5% of a commercial vitamin-mineral mix. Both additives were added inside the wheat bran pellet at a temperature of 62° C. The diet contained 73% dry matter and the following nutritional profile: 12,4% PB, 5,3% de E.E., 28,3% NDF, 43,2% starch, 5,2% ashes and 3,14 Mcal/kg MS ME. Steers did not receive growth-promoting implants during the experiment.

The TMR was offered twice daily at 0800 h and 1600 h in amounts to ensure ad libitum consumption and yield 15% (as-fed basis) orts from the previous feeding. Steers received treatments and the finishing TMR until slaughter.

Initial and final BW were used to calculate average daily gain (ADG) (kg/day) during the experiment. Feed intake was recorded daily for each pen from day 0 until the end of the experiment. Hot carcass weight was measured in the slaughter house.

Results

No treatment differences were detected ($P \geq 0.65$) for initial BW. Treatment differences were detected for final BW (447, 9 and 457,3 kg, C and EX respectively; $P \geq 0.05$), as well as ADG during the experimental period (1,46 and 1,54 kg/d, C and EX respectively; $P \leq 0.05$). Feed intake also differed between C and EX treatments (9,83 and 10,03, respectively; $P \leq 0.05$). Feed efficiency did not differ between treatment ($P = 0.17$). Additionally, no treatment differences were detected ($P \geq 0.39$) for carcass traits, including hot carcass weight and yield.

Conclusions

Inclusion of xylanases (500 UI/g of activity) and Aspergillus oryzae spores ($2x10^4$ UFC/g) have a positive effect on the performance of steers fed with high-energy finishing diets. The use of this additive can be a replacement option for the monensin ionophore without generating detriment to the conversion efficiency.

1442 - COMPARATIVE STUDY OF THE IN VITRO DIGESTIBILITY OF NEUTRAL DETERGENT FIBER IN CORN SILAGES

Author: Luis Diaz, Sharon Gonzales, Juan Reátegui, Jorge Zegarra

Objectives

The research work was carried out in the coastal strip of Arequipa - Peru, Majes irrigation area geographically located at 16°20'00.37" S 72°12'24.85" W. at 1447 m.a.s.l. The objective was to evaluate 03 varieties of corn silage samples: [Insignia 860] (INS), [Dekalb 7500] (DK75) and [Dekalb 399] (DK39), harvested at 110±10 days and preserved in fermented Bunker silos for 30 days.

Material and methods

The nutritional components were analyzed by conventional laboratory methodologies according to AOAC international methods, in vitro digestibility was analyzed by the Ankom Daisy II method. The data were evaluated in a completely randomized design, with three treatments and six repetitions. The in vitro digestibility of dry matter (IVD_{DM}) and neutral detergent fiber (IVD_{NDF}) were evaluated in a completely randomized design with a 3 x 2 factorial arrangement, three varieties of corn and 2 incubation times (30 and 48 hrs).

Results

Differences ($p<0.05$) were observed in the percentage of dry matter, ether extract, ash, neutral detergent fiber, acid detergent fiber, non-fibrous carbohydrates and net lactation energy among the 03 varieties of corn, with the highest value for DK75 with 34.0%, 2.2%, 51% and 1.46 Mcal/kg DM; for dry matter, ether extract, non-fibrous carbohydrates and net lactation energy, respectively. The INS variety obtained the highest value for ash (7.0%), neutral detergent fiber (44.0%) and acid detergent fiber (27.2%). There were no differences ($p>0.05$) in crude protein, lignin, protein insoluble in neutral detergent or protein insoluble in acid detergent between varieties. In the IVD_{DM} evaluation, differences were found ($p<0.01$) both between varieties and between incubation times. The INS variety had the highest IVD_{DM} with 73.3% followed by DK75 with 72.5% without differences between them and DK39 with a IVD_{DM} of 66.9%. In hours of incubation, the IVD_{DM} at 48 hrs was 72.0% and at 30 hrs 69.8% with differences ($p<0.01$) between both. In the IVD_{NDF}, differences were found ($p<0.01$) between incubation times, there is no difference between varieties ($p>0.05$), with values of 53.8%, 52.6% and 50.6% for DK39, DK75 and INS respectively. In incubation hours, the highest IVD_{NDF} was obtained at 48 hours with 54.7% and the lowest at 30 hours with 49.9% with differences ($p<0.01$) between both.

Conclusions

We conclude that the IVD_{NDF} values demonstrated, on average, a greater digestibility of the fiber of the varieties evaluated with respect to the values reported in the literature, recommending its use under the conditions of the coastal strip of Arequipa - Peru.

1469 - Dynamic changes in postprandial plasma free amino acid levels of the hepatic portal, hepatic, and jugular veins in the healthy pre-ruminant calves.

Author: HUEY SHY CHEE , Aiko Kinami Yamamoto, Yoshiyuki Tsuchiya, Yuki Hoshino, Shuji Terui, Atsushi Kimura, Tomomi Kanazawa, Toshihiro Ichijo

Objectives
A first attempt was made to investigate the dynamic changes in postprandial free amino acid (AA) levels over eight time points in plasma, collected from the hepatic portal, hepatic, and jugular veins in pre-weaning calves, to better understand the intrahepatic, pre-and-post-hepatic AA metabolisms.
Material and methods
Nine healthy pre-weaning Holstein male calves were enrolled in the study. The calves were non-surgically implanted with catheters in the hepatic portal vein (HPV) and in the hepatic vein (HP). A series of eight blood samples were taken from the left jugular vein (JV), HPV and HV from each calf at pre, 30, 60, 90, 120, 180, 240, and 360 min after feeding. The analysis of the free AA concentrations in the plasma samples was performed by LC/MS instrumentation.
Results
The calves remained healthy throughout the study and no visual signs of infection or inflammation were observed from results of the blood test and blood chemistry tests. A similar appearance profile with two postpradial peaks for the plasma total free AA (TAA), essential amino acids (EAA), and non-essential amino acids (NEAA) was observed in all three vessels. The postprandial concentrations of individual free AA in the plasma varied markedly from one sampling time to another, and were mainly categorized into three profiles according to the transition patterns of their concentrations. The change over time of most of the individual plasma free AA concentrations ($P<0.05$) was observed more often in the HPV samples than in the HV and JV samples. Except for glycine and hydroxyproline showing close to peak concentrations at feeding, and decreasing constantly between 30 and 360 min after feeding, the concentrations of all other free AA in the plasma were always above their baseline levels at all the sampling time points within 360 min after feeding.
Conclusions
The triple catheterization in calves is practical, and can be utilized to track the uptake profiles of individual plasma free AAs for nutritional physiological studies in a more accurate manner than either a single cannulation or multiple cannulations just before and after feeding. In contrast to the other plasma free AA, glycine and hydroxyproline decreased and were lower than baseline levels after feeding, indicating that the supply of these amino acids in the diet given to the pre-weaning calves in this study was inadequate to meet their needs.

1530 - Effect of the Use of a Lithothamnium calcareum-Based Product on Serum Iron and Hepcidin Concentrations in Holstein Cows

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Objectives

The aim of this study was to compare the effect of supplementation with two different rumen buffers, *Lithothamnium calcareum* and Sodium Bicarbonate, on Iron and hepcidin serum levels in Holstein dairy cows, as well as their correlation with other biochemical parameters.

Material and methods

The experiment was conducted on a commercial dairy farm located in southern Brazil. Thirty-six multiparous Holstein dairy cows with an average of 63.74±18.63 days in lactation and an average milk production of 38.83±9.29 kg were used. The animals were housed in a Compost Barn system and were randomly divided into two groups: *Lithothamnium calcareum* Group (Litho; n=18), which received 0.5% dry matter (DM) of *Lithothamnium calcareum* in the diet (LithoNutri®, Oceana, Jundiaí, Brazil), and Control Group (Con; n=18), which received the equivalent of 1.1% DM of Sodium Bicarbonate daily mixed with the total mixed ration (TMR), following the recommendations of NRC 2001, containing 29.28% starch in the TMR for 60 days of the experimental period. Blood samples were collected by puncturing the coccygeal arteriovenous complex using the Vacutainer system (BD Diagnostics, São Paulo, Brazil) with tubes without anticoagulant and with sodium fluoride on days 0, 21, 42, and 60. Serum iron (sFe), total proteins (TP), albumin (Alb), magnesium (Mg), total calcium (tCa), urea, glucose (Glu), free fatty acids (FFA), and betahydroxybutyrate (BHB) levels were measured using an automatic biochemical analyzer (Labmax Plenno, Labtest®, Minas Gerais, Brazil) with commercial kits following the manufacturer's recommendations (Labtest®, Minas Gerais, Brazil). Hepcidin concentration was measured by ELISA using commercial kits following the manufacturer's recommendations (ELK Biotechnology, Wuhan, China). Dry matter intake (DMI) was measured daily using smart feeders (Intergado®, Minas Gerais, Brazil). Statistical analysis was performed using JMP Pro 17 software (SAS Institute Inc., North Carolina, USA) for sFe and Hepcidin with fixed effects of group, collection, and their interaction. Bivariate correlation analysis was also performed between biochemical parameters, and for correlation with DMI, collection averages were calculated for each collection day (Collection 0 = Day 1; Collection 21 = days 2 to 21; Collection 42 = days 22 to 42; Collection 60 = days 43 to 60).

Results

No differences were found in sFe concentrations (Litho = 142.58±6.41 µg/dL vs. Con =136.15±6.47 µg/dL, p=0.48) and hepcidin (Litho = 1.25±0.08 ng/mL vs. Con =1.10±0.08 ng/mL, p=0.21) between groups. However, positive correlations were found between sFe vs. Alb (r=0.29, p<0.01), sFe vs. Mg (r=0.18, p=0.03), TP vs. tCa (r=0.24, p=0.01), TP vs. Urea (r=0.26, p<0.01), TP vs. BHB (r=0.19, p=0.03), Alb vs. Mg (r=0.21, p=0.02), Hepcidin vs. BHB (r=0.27, p=0.02) and Hepcidin vs Urea (r=0.23, p=0.04). Negative correlations were found between Glu vs. Alb (r=-0.20, p=0.02), Glu vs. tCa (r=-0.20, p=0.02), Glu vs. Urea (r=-0.20, p=0.02), Glu vs. DMI (r=-0.23, p=0.01), and BHB vs. FFA (r=-0.23, p=0.01), while other correlations were not significant.

Conclusions

The inclusion of *Lithothamnium calcareum* in the diets of dairy cows did not result in differences in sFe and Hepcidin concentrations compared to the inclusion of Sodium Bicarbonate. However, some correlations were observed between variables of metabolic importance, indicating potential implications for productive performance.

1537 - Effect of betaine hydrochloride administration in lactating Holstein calves

Author: Toshihiro Ichijo , Atsushi Kimura, Aiko Kinami , Yoshiyuki Tsuchiya, Tomomi Kanazawa, Yuki Hoshino

Objectives
Treatment and prevention of digestive disorders in calves is a major challenge. The betaine hydrochloride formulation used in this study contains betaine hydrochloride (BIOPAIR; TOA BIOPHARMA Co., Ltd, Tokyo) and acid-resistant digestive enzymes, and is used in clinical practice to treat diarrhea in calves. Betaine hydrochloride is hydrolyzed in the stomach to release hydrochloric acid and betaine, and hydrochloric acid lowers the pH in the fourth stomach. Betaine is also used as a feed additive in pigs and poultry that favorably affects digestion and absorption. The purpose of this study was to investigate the effects of betaine hydrochloride on digestion and absorption in clinically healthy calves.
Material and methods
Four clinically healthy Holstein calves were given 10 g of BIOPAIR to the milk replacer two days before. Blood was collected from the jugular vein and hepatic portal vein before feeding and 120 minutes after feeding. A liver biopsy was performed 120 minutes after feeding. General blood test items, blood biochemistry test items, and amino acid concentrations in plasma were measured in blood samples, and the betaine hydrochloride preparation treatment group (administration group) and the control group were compared. Comprehensive gene expression analysis was performed using next-generation sequencing using liver tissue, and the genes of the administration group and the control group were investigated.
Results
Compared to the control group, the area under the glucose concentration curve (AUC) increased and the AUC of NEFA decreased. In addition, the increase in the concentration of amino acids (taurine, proline and tryptophan) before and after feeding changed significantly between the administration group and the control group. The results of the next-generation sequence of the liver showed that the total number of differentially expressed genes was 65. Compared to the control group, the expression levels of 18 genes increased and the expression levels of 47 genes decreased.
Conclusions
Betaine, which is released from betaine hydrochloride, has been recognized as having the effect of maintaining the morphology of the intestinal tract and increasing the efficiency of digestion and absorption of nutrients. In addition, hydrochloric acid lowers the pH in the stomach, which increases the activity of pepsin and may affect the rate of quaternary gastric emptying. Therefore, it was suggested that the digestion and absorption processes may have been affected in the treatment group compared to the control group. In the liver, the expression of the fibroblast growth factor 21 (FGF21) gene was reduced in the treatment group. The administration of betaine has been shown to have the effect of alleviating fat accumulation in the liver in rodents. The expression of FGF21 is said to be involved in this effect, and in this study, the expected lipid metabolism-related effect of betaine was not confirmed by administration two days of betaine hydrochloride. Betaine hydrochloride preparations may have a positive effect on calf digestion by increasing pepsin activity by lowering gastric pH and increasing protein digestion efficiency by suppressing quaterngastric emptying.

1544 - EFFECTS OF PROBIOTICS ON THE BODY GROWTH OF HOLSTEIN DAIRY CALVES OF THE SMALL-SCALE PRODUCTION SYSTEM

Author: MARIO ALFREDO ESPINOSA MARTINEZ, FERNANDO VILLASEÑOR-GONZÁLEZ, LUIS MONTIEL-OLGUÍN, GERMÁN BUENDÍA-RODRÍGUEZ, JOSE RUVALCABA-GÓMEZ, MARIA DENISSE MONTOYA-FLORES, EDITH ROJAS-ANAYA

Objectives

To evaluate the effects of probiotics on the body growth of Holstein dairy calves of the small-scale production system

Material and methods

The study was conducted in the region of Los Altos de Jalisco, México (20º49'01"N, 102º43'59"W). Dairy calves (n=24) were registered from birth to eight weeks (60 days) of age at two dairy farms with characteristics of the small-scale production system in Mexico. At six, 13 and 20 days old, a group of calves (PROBIO, n=10) received a probiotic dose (1.0e9 CFU/kg weight) that contained two *Lactobacillus* strains in milk replacer. A second group of calves (CONTROL, n=14) received no probiotics. The body weight and withers height of the calves were measured at birth, four and eight weeks of age and daily weight gain and daily height gain at four and eight weeks of age were estimated. The body weight was estimated using a heart girth tape specifically designed for Holstein dairy calves and the withers height was obtained using a somatometric ruler. Analysis of variance considered treatment (PROBIO vs CONTROL) and the dairy farm as block in the statistical model. A value of $P<0.05$ was considered of statistical significance.

Results

The results showed no differences ($P>0.05$) in body weight (41.5±3.0 kg) and withers height at birth (80.7±0.6 cm) between groups. Additionally, the use of probiotics did not show effect ($P>0.05$) on the daily weight gain at four weeks (0.346±0.04 vs 0.322±0.05 kg, for CONTROL and PROBIO groups, respectively) or at eight weeks (0.575±0.05 vs 0.517±0.05 kg) nor on daily height gain of the calves at four weeks (0.158±0.03 vs 0.116±0.04 cm, for CONTROL and PROBIO groups, respectively) or eight weeks (0.199±0.02 vs 0.159±0.02 cm). Lack of differences between groups of calves could be due to factors as the strains used, dosage or the number of doses administered.

Acknowledgments: INIFAP SIGI project 1034034810.

Conclusions

The combination of two strains of *Lactobacillus* used in this study did not show effects on the body growth of Holstein dairy calves from birth to weaning.

1586 - ASSOCIATION OF FEED EFFICIENCY AND ULTRASOUND-MEASURED CARCASS TRAITS IN SIMMENTAL AND SIMBRAH CATTLE

Author: René Calderón-Chagoya, Moisés Montaño-Bermúdez, Vicente E. Vega-Murillo, Ángel Ríos-Utrera, María Denisse Montoya-Flores, Carlos Hernández-López, Guillermo Martínez-Velázquez

OBJECTIVE
The aim of this study was to analyze the association between feed efficiency and carcass traits measured by ultrasonography in Simmental and Simbrah cattle.
MATERIAL AND METHODS
The study utilized information from 76 animals, consisting of 44 Simmental (19 females and 25 males) and 33 Simbrah (10 females and 23 males). On average, the animals were 272.72 days old at the beginning of the measurements. The feeding trial was conducted in facilities equipped with the GrowSafe® system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada), spanning a duration of 83 days. Animals were weighed at the start and end of the trial. Average daily gain (ADG) and dry matter intake (DMI) were calculated to determine feed-to-gain ratio (F:G).
Residual feed intake (RFI) was calculated as the disparity between the actual DMI of each animal and its predicted DMI based on its metabolic weight ($kg^{0.75}$) and ADG, following the procedures outlined by Arthur et al. (2001). RFI was classified into three categories: low, medium, and high, utilizing 0.5 standard deviations above and below the mean to group the data (Nkrumah et al., 2007).
Real-time ultrasonography was employed to measure longissimus muscle area (LMA), intramuscular fat (IMF), and the back fat thickness (BFT) and rump fat (RUF). Feed efficiency traits (F:G and RFI) and carcass traits (IMF, LMA, BFT, and RF) were analyzed using the GLM and CORR procedures in the SAS program. Fixed effects included in the analysis were breed, gender, and RFI classification.
RESULTS
ADG exhibited a positive correlation ($P<0.05$) with DMI ($r=0.56$) and a negative correlation with F:G ($r=-0.61$); no significant correlation was found with other traits, including RFI. DMI showed positive correlations with F:G ($r=0.30$), RFI ($r=0.53$), and RUF ($r=0.27$). F:G was positively correlated ($P<0.05$) with RFI ($r=0.45$), BFT ($r=0.33$), and RUF ($r=0.35$). IMF did not show any correlation with other studied traits in this work. LMA, BFT, and RUF were correlated with each other (r between 0.35 and 0.74).
The RFI category impacted DMI, F:G, and RFI ($P<0.05$). For DMI and RFI, the low, medium, and high RFI groups were statistically different from each other. For F:G, the high RFI group differed from the low and medium food intake groups. The breed effect was significant for BFT and RUF ($P<0.05$); Simbrah exhibited higher values, 0.16 and 0.20 mm for BFT and RUF, respectively, compared to Simmental, which had 0.12 and 0.13 mm for BFT and RUF, respectively. Sex had a significant effect on ADG, F:G, BFT, and RUF ($P<0.05$); females showed lower ADG compared to males but expressed higher F:G, BFT, and RUF.
CONCLUSIONS
Differences were observed through the high, medium, and low classification of RFI. In contrast to F:G, RFI is independent of carcass traits, as no significant correlation was observed.

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1588 - FIRMICUTES TO BACTEROIDETES RATIO AND THEIR IMPACT ON RESIDUAL FEED INTAKE AND METHANE EMISSIONS IN SIMMENTAL AND SIMBRAH CATTLE

Author: María Denisse Montoya-Flores, René Calderón-Chagoya, Moisés Montaño-Bermúdez, Vicente E. Vega-Murillo, Ángel Ríos-Utrera, Carlos Hernández-López, Guillermo Martínez-Velázquez

Objectives

The objective of this study was to analyze the correlation between Firmicutes to Bacteroidetes ratio with residual feed intake and methane emissions in Simmental and Simbrah cattle.

Material and methods

The study employed data from 76 animals, including of 44 Simmental (19 females and 25 males) and 33 Simbrah (10 females and 23 males). The average age of the animals at the commencement of measurements was 272.72 days. The 83 day feeding trial took place in facilities equipped with the GrowSafe® system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Animal weighing occurred at both the initiation and culmination of the trial.

Residual feed intake (RFI) was determined as the difference between each animal's actual dry matter intake (DMI) and its predicted DMI based on metabolic weight ($kg^{0.75}$) and average daily gain (ADG), in accordance with the protocols delineated by Arthur *et al*. (2001).

Methane emission factors were computed using the Intergovernmental Panel on Climate Change (IPCC) Tier 2 approach, with gross energy intake (MJ/d) derived from the IPCC prediction equation and a methane conversion factor of 6.3 (Berdos *et al.*, 2023).

Taxonomic phyla abundances of Firmicutes and Bacteroidetes were determined through the extraction of DNA from rumen content obtained from each animal. Sampling of the rumen content involved the use of a stomach tube connected to a mechanical pumping unit, and the collected samples were stored in sterilized containers.

Subsequent analyses of taxonomical phyla abundances, including Firmicutes, Bacteroidetes and the Firmicutes to Bacteroidetes ratio with residual feed intake and methane production were carried out utilizing the CORR procedures within the SAS program.

Results

The study revealed a significant positive correlation of 0.56 ($P<0.0001$) between Firmicutes and Bacteroidetes, suggesting a noteworthy association between these microbial populations. Notably, the Firmicutes to Bacteroidetes ratio showed no discernible link with residual feed intake and methane production. However, it is important that Firmicutes exhibits a negative correlation of 0.24 ($P<0.05$) with both residual feed intake and methane production, suggesting a potential influence on these traits.

Importantly, no significant variations were observed when analyzing these traits in relation to breed and sex, implying that the identified relationships are not significantly impacted by such factors.

Conclusions

A significant positive correlation exists between Firmicutes and Bacteroidetes. The Firmicutes to Bacteroidetes ratio shows no link to feed efficiency and methane production, but Firmicutes correlates negatively with these traits. These findings contribute to our understanding of the complex interplay between microbial populations and key physiological traits in the context of feed efficiency and methane production.

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1601 - Effect of quebracho level and diet type on in vitro ruminal fermentation and methane production

Author: Valeria Guadalupe Jimenez-Arévalo, Atmir Romero-Pérez, Jose Talamante-Gomez, Clauda Márquez-Mota, Maria Fernanda Vazquez-Carrillo, Luis Corona-Gochi

Objectives
Quebracho extracts, as a source of tannins, have been used as an additive in diets for ruminants to reduce the degradation of ruminal protein and methane. However, their effect has been very variable since it depends on the source, dose of tannins, and type of diet. Therefore, the objective of the study was to evaluate the effect of four doses of a commercial product based on quebracho that contains tannins and three types of diets: Feedlot, Dairy Cattle, and Grazing, which correspond to low, medium, and high level of forage respectively, using the in vitro gas production technique in ruminal fermentation, dry matter digestibility and methane production to determine its potential use as a greenhouse gas mitigation strategy in ruminants.
Material and methods
An in vitro trial was carried out in a completely randomized block design with a factorial arrangement of 4 (dose: 0, 0.25, 0.50, and 0.75 % of [PE, Protein Enhancer®]) x 3 (diet types: low [LFD, medium (MFD) and high in forage (HFD). The fermentation of each treatment was carried out in triplicate in 120 mL amber antibiotic bottles with a 20 mm nozzle, introducing the substrate (0.5g) previously deposited in nylon bags. (size of 4.5 × 11 cm and 24 µm pore size) at the constant weight and 60 mL of a medium and rumen fluid mixture in an anaerobic environment. In three independent runs (blocks). The bottles were sealed and incubated in a water bath at 39°C with lateral agitation at 30/min. The gas pressure (PSI) was recorded at 1, 2, 3, 6, 9, 12, 18, 24, 30, 36, 48, 60 and 72 hours after -incubation. Gas pressure data were converted to gas volume using an equation developed under laboratory conditions. Subsequently, pH was measured, and subsamples of 4 mL of ruminal fluid plus 1 mL of metaphosphoric acid (HPO3) were taken. 25% for subsequent analysis of volatile fatty acids (VFA) by gas chromatography. The in vitro degradation of dry matter (DIVMS) was calculated. For the determination of methane, the Crowcon Gas-Pro detector (Oxfordshire, UK. W365164/01-001) was used, and for CO2, the Crowcon Gasman gas detector (527165/03-008) was used from the bottles with the gas collected during the 72 hrs of incubation. Ammoniacal nitrogen was determined using a hybrid multimodal microplate reader (SYNERGY™ H1). The data were analyzed with the MIXED procedure of SAS, and the comparison of means was analyzed with Tukey and with orthogonal polynomials for doses (Cochran and Cox, 1992). The differences were considered significant when the value of $P<0.05$ and trends $P<0.10$.
Results
Interaction ($P < 0.05$) was observed between diets and PE levels for % CH4, where for the diet with a high level of forage and the highest level of PE (0.75%), it was lower ($P < 0.05$) by 30% in comparison with the control diet of dairy cattle. On average, the mL CH4/g DM were 83.3, 63.6, and 35.3 for LFD, MFD, and HFD, respectively. As the dose of PE increased, rumen pH and N-NH3 decreased (linear effect, $P < 0.05$), and the total VFA concentration increased ($P < 0.05$).
Conclusions

Methane production is lower (30%) with the grass-based diet and the highest level of quebracho-based product with condensed tannins, compared to the dairy cattle diet with 0% condensed tannins. The diet for feedlot cattle is more digestible and, therefore, produces more gas and a higher concentration of CH_4, in contrast to the grass-based diet, which produces less methane. As the dose of the product with quebracho increases, rumen pH and N-NH_3 decrease, but the total VFA concentration increases. It is necessary to carry out in vivo trials, particularly in grazing cattle, to evaluate the effect of the quebracho-based product on performance and *in vivo* methane emission.

This study was partially funded by PAPIIT-DGAPA-UNAM (grant IN212523).

1652 - Colostrum whey and antioxidants as a supplement to improve performance and health of male dairy calves.

Author: Jessica Pereira, Joao Costa , Joao Lovatti

Objectives

Enhancing calf health during the preweaning and postweaning period is crucial for animal development, short- and long-term productivity, and to reduce antimicrobial use in the dairy enterprise. Bovine colostrum whey protein has several functional and biological activities, such as immunomodulatory and antioxidation activities, and inhibition of DNA oxidative damage. The objective of this study was to determine if supplementing milk replacer with colostrum whey proteins has beneficial effects on disease occurrence and performance during the rearing period of dairy calves.

Material and methods

Holstein male calves (n = 124) were enrolled in an 84-day randomized controlled trial divided in three periods: preweaning (d1 to d42), weaning (d43 to d63), and postweaning (d64 to d84). Upon enrollment, calves were assigned to one of three treatments: no supplement (CW0: Placebo; n = 41; BW = 47.16 ± 3.38 kg), 57 g/head/day for 7 days (CW7; n =41; BW = 46.74 ± 3.48 kg), or 57 g/head/day for 14 days (CW14; n = 43; BW = 46.80 ± 2.88 kg) of colostrum whey powder. The supplement was blended with milk replacer (26% CP, 17% fat; 130 g/L) and fed to calves twice daily. Calves were individually housed and had unlimited access to water and a pelletized calf starter (20% CP). Calves were fed 4 L/d of milk replacer divided into two equal meals until d7; milk feeding plan increased until d41 (8 L/d), and on d42, milk allowance was gradually reduced (2.5 L/d) until completely weaned on d63. Calf starter intake and body weight were recorded weekly until d84. Calves were health scored daily for BRD and diarrhea. A BRD case was defined as a BRD score ≥ 5, and a diarrhea case was defined as a watery fecal consistency for 2 d. Cases were treated following standard far procedures, and number of treatments were recorded. A mixed linear regression model (PROC MIXED) determined the effect of treatment on calf starter intake and average daily gain (ADG) for each period and the total experimental period. The model included the fixed effects of serum total protein concentration at arrival, milk consumed during the experimental period, enrollment body weight, treatment, number of disease cases (BRD and/or diarrhea), and their interaction. Number of therapeutic interventions were analyzed using GENMOD procedure of SAS, where the model included fixed effects of number of disease cases of BRD or diarrhea, serum total protein concentration at arrival, and milk consumed during the experimental period.

Results

Overall, the average calf starter feed intake from d0 to d84 was 1.16 ± 0.04, 1.14 ± 0.04, and 1.10 ± 0.04 kg/d, for CW0, CW7 and CW14, respectively, with no treatment effect for the total experimental period (d0-d84; $P = 0.41$), or any of preweaning, weaning or postweaning periods (P = 0.53, P = 0.68, and P = 0.43 respectively). However, there was an interaction between disease bouts and treatment for calf starter intake ($P = 0.01$), where calves that did not receive a supplement (CW0, placebo) had less calf starter intake (- 0.17 ± 0.9 kg/day; $P = 0.04$) during a disease bout compared to supplemented calves who showed no change in intake. Overall, ADG from d0 to d84 was 0.81 ± 0.02, 0.83 ± 0.02, and 0.78 ± 0.02 kg/d, for CW0, CW7 and CW14, respectively, with no

treatment effect for the total experimental period (d0-d84; *P* = 0.40), or any of the preweaning, weaning and postweaning periods (P = 0.63, P = 0.74, and P = 0.97, respectively). No differences were observed in the incidence of treatments, diarrhea, or BRD between treatments (*P* = 0.35, *P* = 0.64, *P* = 0.61). Overall, 50.79% [HN1] and 26.98% of all calves were treated at least once for diarrhea and BRD, respectively.
[HN1]Should this just be one decimal..

Conclusions

Future research should investigate the effects of colostrum whey with antioxidants on blood metabolites and gut permeability during the calf-rearing period.

1672 - In vitro evaluation of chia seed (Salvia hispanica) potential to reduce methane emissions in ruminants

Author: Rebeca Monserrat Franco Hernández, Atmir Romero-Pérez, Luis Corona Gochi, José Moises Talamantes Gómez, Claudia Cecilia Márquez Mota

Objectives

Reduction of enteric methane (CH_4) emissions from ruminants is of great importance because CH_4 is a potent greenhouse gas that contributes to global warming and ruminant animals contribute significantly to this problem through their natural digestive process. Previous studies have shown that the use of supplementation with oilseeds such as sunflower, canola or linseed seeds, works to reduce CH_4 emissions. Thus, the main objective of this study was to evaluate an ancient seed native to Centroamerica with high fat content, the chia seed (*Salvia hispanica),* to determine their potential to inhibit metanogenesis and its effects on rumen fermentation *in vitro*. Also, to evaluate the addition of chia seed using in two different forages, alfalfa and corn stover.

Material and methods

The experiment was arranged as a completely randomized block design with a 3 × 2 factorial arrangement of treatments, including 3 presentations of chia (crushed, ground and oil) and two types of forage (alfalfa and corn stover). The oil sources were added to the substrate to provide a level of 3.82% added fat (DM basis) and a total fat level of 8 and 7.3% for alfalfa and corn stover respectively. At the same time, a control without oil and a positive control with linseed oil were included. Three independent batch culture runs were conducted carried out. A total of 0.5 g of substrate was weighed into nylon filter bags and placed in amber glass bottles. The rumen liquid used as inoculum was obtained from two cows before the morning feeding. Bottles were filled with a combination of inoculum and buffer solution and incubated in a water bath for 72 h at 39°C. Total gas production (TGP), CH_4 production, dry matter digestibility (DMD), and volatile fatty acid concentration (VFA) were evaluated. Data were analyzed with the MIXED procedure of SAS using the Tukey instruction for mean comparison.

Results

The type of fat × forage interaction was not significant for total gas produced (mL; $P = 0.51$). However, all different fat sources reduced ($P < 0.01$) total gas production by 23.37% on average, mainly when corn stover was used as forage ($P = 0.03$). Methane production (mL) decreased ($P < 0.01$) up to 37.56% with all different presentations of chia, particularly when alfalfa was used. Consequently, a reduction (11.17%) in total VFA concentration (mMo) was also observed with chia addition ($P < 0.01$) where the corn stover had a slightly higher VFA concentration as compared with alfalfa ($P = 0.01$). The concentration of propionate increased with the use of linseed oil ($P = 0.03$). Additionally, the propionate concentration was higher when alfalfa was used as forage ($P < 0.01$). The acetate concentration decreased with both the use of linseed oil and the use of chia oil ($P = 0.03$) and was lower for alfalfa as compared with corn stover ($P < 0.01$). As expected, the reduction of total gas production was associated to a reduction of DMD. The forage x fat interaction was significant ($P < 0.01$) for DMD. The addition of different fat presentations reduced DMD by 16.87% when using alfalfa and by 11.53%, when using corn stover.

Conclusions

All different chia presentations evaluated decreased methane emissions by ~30% on average when compared to the negative control using both, alfalfa and corn stover. Typically, dietary fat addition is associated with moderate reductions in methane emissions. However, in the current study, the mitigation potential was high with consequent reduction in dry matter digestibility. Therefore, including a lower level of dietary fat from chía seed might be an option to reduce methane production without affecting digestibility.

1703 - IN SITU DEGRADABILITY AND IN VITRO METHANE PRODUCTION OF A SHEEP DIET SUPPLEMENTED WITH ESSENTIAL OILS AND CALCIUM MALATE

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Objectives
The objective of this research was to measure the ruminal degradability of dry matter, fiber fractions and *in vitro* biogas production of diets treated with essential oils (EO), calcium malate (CM) and their combination (EO+CM).
Material and methods
The nylon bag technique was used to determine *in situ* disappearance and ruminal degradability parameters with four sheep fistulated in rumen and distributed in a Latin Square design.
Results
The ruminal incubation periods were 0, 3, 6, 9, 12, 24 and 48 h. The treatments were the basal diet (BD); BD with EO 150 g t^{-1}; BD with CM 2.5 kg t^{-1} and BD with EO 150 g t^{-1} and CM 2.5 kg t^{-1}. The *in vitro* methane and carbon dioxide production was carried out for 24 h. The addition of additives did not modify ($P > 0.05$) the disappearance of dry matter from 0 to 48 h, although the neutral detergent fiber disappearance was higher ($P < 0.05$) at 3 h of incubation in EO diet. The degradability parameters (i.e. a, b and c) and molar concentration of acetate, propionate, butyrate, were not affected ($P > 0.05$) by the additives. The effective degradability of the potential degradable fraction of dry matter was higher ($P < 0.05$) in EO diet compared to CM or EO+CM diets, but similar ($P > 0.05$) to BD. The EO diet reduced ($P < 0.05$) *in vitro* methane production and increased ($P < 0.05$) carbon dioxide production compared to EO+CM diet.
Conclusions
These data indicate that EO, but not their combination with MC, improve the effective dry matter degradability and reduce methane production *in vitro*.

1707 - Transition cow health and management in pasture-based dairy herds: Results of a farmers' survey

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Objectives
The transition period may be defined as the few weeks before and after calving. While much has been published about this period in confined dairy cows, less has been published on pasture-based dairy cows. This study aimed to describe dairy farmers' perceptions of the transition period, disease incidence and management strategies in order to inform and guide future research.
Material and methods
An online survey, consisting of 19 questions was distributed via text message amongst 3,899 Teagasc (The Agriculture and Food Development Authority in the Republic of Ireland) dairy advisory farmer clients.
Results
In total, 525 farmers responded to the survey (response rate = 15.3%). Fresh cow diseases (e.g. milk fever, retained placenta, metritis, displaced abomasum, ketosis) were ranked to be of highest importance by nearly half of respondents (49%; 252/510). Freshly calved cows' health was identified as critical and a good transition to set the cow up for lactation (86%; 452/523). The incidence of disease was reported to be highest in three risk groupings: fresh cows (first 3 weeks after calving; 58%; 299/519), in multiparous cows (52%; 266/513) and in cows calving at the end of the calving season (late calvers; 48%; 245/510).
Most respondents reported treating 1-6% of their herd for milk fever (66%; 342/521) or retained placenta (66%; 343/518), while <1% of the herds were reported to be treated for grass tetany (hypomagnesemia) (83%; 419/507), ketosis (73%; 368/506) or displaced abomasum (71%; 373/521) in an 'average' year. A small but considerable proportion of respondents indicated that both milk fever and subclinical hypocalcaemia were a significant problem (regularly treating severe cases with some cows lost/culled) or a routine problem (regularly treating cows to control issues) in their herds (milk fever: 16% and subclinical hypocalcaemia: 9%). Farmers with small herds (<60 cows) commonly reported not knowing what subclinical hypocalcaemia (26%; 19/74) or subclinical ketosis (36%; 26/72) were (for larger herd owners: 20% (85/434) and 30% (128/430), respectively). Farmers commonly kept records of antibiotic treatments for diseases such as displaced abomasum (55%; 285/519), retained placenta (50%; 258/516) and metritis (39%; 199/505). However, some farmers, most frequently with small herds, also reported to keep records of antibiotic treatments for milk fever (41%; 30/74), ketosis (32%; 23/73) and grass tetany [21%; 15/71; for other herds: 21% (91/429), 23% (92/408) and 14.6% (62/424), respectively].
Correct management during the dry period was regarded as essential for future health and performance by 90% (467/521) of respondents. Body condition monitoring was the most commonly reported dry cow management strategy by all farmers (73%; 365/497). However, for split-calving herds (those calving in spring and autumn), provision of feed sources other than silage was the most commonly reported dry cow management strategy [64% (43/67); for spring-calving herds: 43% (184/428)]. Dry cow management

in >1 group (65%; 96/148) and magnesium supplementation (63%; 93/148) were most frequently reported by large herd owners [for other herds: 50% (170/343) and 45% (153/343), respectively]. Regarding fresh cow management, keeping freshly calved cows indoors for a period post-calving (68%; 331/487) and calcium supplementation of high-risk cows (56.7%; 276/487) were the most commonly reported strategies by all farmers. However, the former was more frequently reported for spring- (72%; 298/416) than for split-calving herds (42%; 28/67). Large and spring-calving herd owners were also more likely to report implementing once-a-day milking for a period after calving and magnesium supplementation to fresh cows compared to small and split-calving herd owners.

Conclusions

The results from this survey suggest that future research in transition cow health and management in pasture-based herds should focus on milk fever, dry cow body condition monitoring and fresh cow housing and calcium supplementation.

1731 - Mycotoxin adsorbing products effectiveness evaluation in lactating dairy cows feed using in vitro model

Author: Samanta Arlauskaitė , Vita Baliukonytė

Objectives

Each year dairy cattle feed contamination with mycotoxins is causing progressively more health issues and loss of productivity. Notwithstanding the fact that more than 500 types of mycotoxins have been identified, the main mycotoxins, found in cattle feed are aflatoxin B_1 (AFB_1), deoxynivalenol (DON), zearalenone (ZEN) and fumonisin B_1 (FB_1). Due to their mass distribution as well as difficulty in removal, mycotoxin adsorbing products in dairy cattle feed became widely used. The aim of this study was to evaluate commercial mycotoxin deactivation of feed additives using *in vitro* model of the cow's rumen at at pH 5.5 and 6.8, after zero, four and a half, and nine hours after contact.

Material and methods

The research was conducted in the Lithuanian University of Health Sciences, Veterinary Academy, Department of Food Safety and Quality and the Mycotoxicology Laboratory in 2022. Total of 4 different lactating cow's feed sample were collected from various farms in Lithuania. AFL B1, DON, ZEA, OTA concentrations were evaluated in selected samples by ISO 21527-1:2008 standart "Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0,95". In vitro model was made using lactating cow's rumen liquor and buffer solution. Two mycotoxin deactivating products: Mycofix® Plus 3.E (Biomin Holding GmbH, Getzersdorf, Austria) and ZeoFeed® (Zeocem) were assorted to the feed mixtures to estimate their effictiveness at pH 5.5 and 6.8, 0, 4.5, and 9 hours after contact.

Results

The findings of this study shows that AFL B1 and DON were found in 50 %, ZEA in 75 % of all collected samples, but only the recommended maximum concentration of ZEA, defined by EU commission recommendation 2006/576/EC, was exceeded. Research with *In vitro* cow's rumen model has shown that feed additive ZeoFeed® resulted in total reduction of DON and ZEA in feed samples after four and a half hours, while Mycofix® Plus 3.E reduced a 100% of the sum of ZEA, AFL B1 and DON after nine hours.

Conclusions

According to our research and its results it has been found that both selected products significantly reduce mycotoxins concentration: after 9 hours Mycofix® Plus 3.E decreased 100 % of DON and AFL B1 ($p<0,01$), but it was less effective while decreasing ZEA concentration after same period of time. After 4,5 hours ZeoFeed® has reduced 100 % of DON and ZEA ($p<0,01$), yet this product was not beneficial with pH 5.5. Further research is needed in order to compare effectiveness of these mycotoxin deactivators.

1797 - DIGESTIBILITY, FERMENTATION PARAMETERS AND SHORT CHAIN FATTY ACIDS PRODUCTION UNDER IN VITRO CONDITIONS OF DIETS WITH PECAN SHELLS FOR FATTENING LAMBS

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Objectives

The aim of this study was to evaluate *in vitro* the dry matter digestibility, parameters of fermentation and short chain fatty acids (SCFA) production of diets formulated with pecan shells for fattening lambs.

Material and methods

This study was carried out in the Animal Nutrition laboratory of the Autonomous University of the State of Hidalgo. Firstly, the pecan shells (Ps) were analyzed (proximal chemical and cell wall-analysis) to formulate four diets (isonergetic, 2,79 Mcal/kg DM; isonitrogenated, 8,8% CP). The feeder lamb diets included on a dry basis increasing amounts of pecan shells: control (Ps0, no pecan shell), 4% (Ps4), 8% (Ps8), and 12% (Ps12). *In vitro* fermentation was conducted using the technique proposed by Theodorou et al. (1994). Rumen fluid was collected from two cattle (450.0 kg BW) with ruminal cannulas (Colegio de Postgraduados, campus Montecillo) managed in accordance with the regulations for the care and use of laboratory animals (NOM-062-ZOO -1999). For in vitro incubation, 500 mg of the diets were ground to a 1 mm particle size were placed in a flask with 40 mL of a mixture consisting of 225 mL of buffer solution, 225 mL of macromineral solution, 100 mL of ruminal fluid and 450 mL of distilled water per 1000 mL of mixture. Additionally, 100 µL of a micromineral solution and 2 mL of a reducing solution were added for every 60 mL of mixture. The container was closed and sealed for incubation in a water bath at 39°C. The volume of gas produced was recorded by 72 h of incubation. At the end of the incubation period, the pH of the medium was measured. Then, 0.8 mL of the medium was recovered and 0.2 mL of metaphosphoric acid (25%) was added for the quantification of SCFA by gas chromatography (AutoSystem XL, PerkinElmer Instruments; capillary column, 30 m x 0.53 mm x 1, 0 µm; PN-125-3232, Agilent-Technologies). The working conditions were injection, 1 µl; injector temperature, 190 °C; oven temperature, 80°C, for one minute, increasing by 15°C each minute to 200°C. The kinetics of gas production were analysed using the model proposed by Schofield and Pell (1995):

$$Va=V/1+e2\text{-}4S(t\text{-}L)$$

Where: Va corresponds to the gas volume at time t; V, maximum volume ($mL^{-1}g^{-1}$ DM); S, gas production rate (h^{-1}); L, lag time (h). The parameters were estimated using the NLIN procedure of SAS® (SAS Institute Inc., 2012) through an iterative non-linear regression process.

Results

The pecan shells (Ps) had higher contents (g^{-1}100gDM) of NDF (62.55±0.16), ADF (58.29±0.19), lignin (18.64±0.07) and ethereal extract (24.97±0.06); but low values of crude protein (5.23±0.37) and IVDMD (7.8±2.2). However, the inclusion of Ps in the diets did not affect (P>0.05) the IVDMD (79.27±6.3), pH (6.62±0.14), nor the S (0.13±0.02) and L (-1.29±0.63) parameters of the gas production kinetics. In contrast, the inclusion of

pecan shells affected (P<0.001) the V parameter, with the highest value for Ps8 (537.8 mL^{-1} g^{-1} DM), followed by Ps12 (498.3 mL^{-1} g^{-1} DM). The Ps0 diet had the highest total SCFA production (76.8 mM), while the Ps8 diet had the lowest (69.5 mM). Ps0 had the highest concentrations (P<0.05) of butyrate (10.7 mM), propionate (16.3 mM), isobutyrate (1.68 mM), valerate (2.05 mM) and isovalerate (2.81 mM), while diet Ps8 had the lowest (P<0.05). Acetate production (41.7±0.96) was not affected (P<0.05) by the inclusion of Ps in the diets. The lowest (P<0.05) acetate/propionate and acetate/butyrate ratios were observed for Ps0 (2.66 and 4.03 mM/mM, respectively), contrary the highest values were observed for Ps12 (2.8 and 4.5 mM/mM, respectively).

Conclusions

The results of the present study show that the inclusion of up to 12% pecan shells in the rations of fattening lambs does not affect the DIVMS, pH, L and S parameters of gas production kinetics. However, including 8% Ps decreased total and individual SCFA production, while the acetate/propionate and acetate/butyrate proportions increased with 12% Ps inclusion. Further *in vitro* and *in vivo* studies are needed to determine an optimal level of pecan shell inclusion for lamb finishing diets.

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1801 - Effect of Dietary Supplementation in Lambs on Productive Performance, Antioxidant Levels, and Methane Emissions: An Analytical Review and Meta-Analysis

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Objectives
Selenium (Se) plays a key role in maintaining cell membrane integrity and modulating antioxidant status. The aim of this study was to conduct an analytical review to determine the overall effect of Se supplementation on production performance, antioxidant activity and methane (CH_4) emissions.
Material and methods
A systematic literature search was conducted to answer the following PICO question: What is the effectiveness of Se supplementation on productivity, antioxidant status, and CH_4 emissions in lambs compared to an unsupplemented group? The search was performed by five independent experts to avoid bias using the scientific databases PubMed, Google Scholar, Web of Science, Springer, and PRIMO-UAEH. Only articles that provided an effect size measure, variability measure, sample size, and randomization of the procedure were included. The effect size was calculated using both standardized mean difference (SMD; Hedges 1981) and raw mean difference (RMD), with the latter allowing for interpretation of the effect size in the original units of measurement. The study considered several dependent variables, including average daily gain (ADG; kg/d), dry matter intake (DMI; kg/d), feed conversion ratio (FCR; kg/kg), glucose (md/dl), glutathione peroxidase (GPX), and CH_4 emissions. To conduct the meta-analyses, we used the 'meta' package (Balduzzi et al., 2019) in R statistical software. We quantified heterogeneity using the I2 index. For response variables that exhibited significant heterogeneity (I2 > 50%), we constructed mixed-effect models (meta-regression analysis) using the 'Metafor' package to investigate the sources of heterogeneity. The covariates included were breed, source (organic, inorganic, Se-yeast, and nano-Se), dosage, lamb body weight, and age (in months).
Results
A total of 26 studies were used in the current meta-analysis. The results indicate that Se supplementation increases ADG (RMD=+0.022 kg/d; P=0.0001) and reduces FCR (RMD=-0.87 kg/kg; P=0.0005) compared to the unsupplemented lambs. However, there was considerable heterogeneity in the ADG results (I2= 56.4%), which was mainly explained by the Se source, with a higher positive effect, based on the meta-regression coefficients, shown by the nano-Se source (+1.2**). Likewise, supplementation with Se-yeast (+19.18**) and organic Se (+19.96**) had a greater positive effect on FCR. The supplementation did not have any significant effect on DMI (RMD=+0.004 kg/d; P=0.44) and glucose levels (RMD=+1.2 mg/ml; P=0.23) of lambs. The articles used in this study reported GPX as either hepatic activity or its concentration in plasma, serum, muscle, or erythrocytes. Therefore, it was not possible to calculate the RMD and the effect size for GPX outcome was reported only as SMD. The study found a positive effect of GPX with Se supplementation (SMD=+1.56; P=0.0001) compared to the control group. CH_4 was

expressed as SMD due to non-harmonizable units of measurement. The supplementation of Se did not affect CH_4 emissions (SMD=+0.19; P=0.53).

Conclusions

Se supplementation in lamb diets improves productive performance, increasing DMI and reducing FCR, which could be partly explained by the better antioxidant status promoted by the higher GPX activity or concentration. However, the level of response to Se supplementation is dependent mainly of their source, with better responses showed by Se-yeast and nano-Se.

REPRODUCTION

1020 - INJECTABLE TRACE MINERALS, STRATEGIC ADMINISTRATION BEFORE FIXED-TIME ARTIFICIAL INSEMINATION IN BEEF CATTLE IN BRAZIL

Author: Marcello Guadagnini Guadagnini, João Victor da Silva Teodoro, Julio Prado, Eithna O'Leary, Gumercindo Franco

Objectives

Trace minerals play an important role in cattle immunity, health and performance. Copper, Selenium, Zinc and Manganese deficiencies are known to negatively influence beef cattle reproduction. Delivery of supplemental trace minerals using an injectable solution has been demonstrated to be a reliable means of achieving adequate trace-mineral status. The aim of the present work is to summarize fertility results obtained in Brazilian beef cattle injected with a combination trace mineral solution before fixed-time AI, compared with non-treated control animals.

Material and methods

Seven Nellore cattle farms in Brazil were enrolled for this study, one in the Goiás state and 6 in the Mato Grosso do Sul state. Overall, 1643 Nellore cows kept in tropical pasture were evaluated. In each farm, between December 2022 and April 2023, non-pregnant cattle were randomly assigned to treatment and control groups. Treatment group (MM) received one subcutaneous injection (1mL/100 kg body weight) of a trace mineral solution containing Zinc (60 mg/mL), Manganese (10 mg/mL), Copper (15 mg/mL) and Selenium (5 mg/mL) [Multimin 90®] 11 days before timed AI. Control animals remained untreated (CON). Cattle were synchronized for fixed-time AI using estradiol and a progesterone-releasing intravaginal device. Parity of enrolled animals was similar in each farm among treatment groups and was related to the availability of non-pregnant animals at that time of year, which represents the mid-end of the breeding season.Body condition score (BCS) was assessed on a 1 to 5 scale at the start of the synchronization protocol for 1485 animals. The reproductive outcome was assessed by performing ultrasound pregnancy checks 30 days after the insemination.Pregnancy diagnosis outcome was treated as a categorical variable (pregnant vs open) and the difference between treatment groups was first assessed with a simple logistic regression analysis. Moreover, in order to account for other different variables, odds of a positive pregnancy diagnosis were calculated using a multiple logistic regression. Then, animals were divided into 2 categories according to BCS (lower than 3 and greater or equal to 3) and odds of a positive pregnancy diagnosis were calculated by category, aiming to better understand the impact of injectable trace mineral supplementation on cattle, differing in BCS. Significance and tendency levels were set at p value<0.05 and <0.10.

Results

350 heifers, 206 first lactation cows and 1087 second lactation and greater cows were included in the analysis. 816 animals were in the MM group and 827 animals in the CON group. Parity composition of each treatment group did not differ (p=0.6). BCS at the beginning of the synchronization protocol did not differ between MM and CON cattle (2.64 vs 2.67 p=0.21). Overall pregnancy percentage was significantly higher in the MM group (50.61%; n=413) compared to CON group (44.38%, n=367) (p=0.01). When

accounting for farm, BCS, parity and the interaction between treatment and BCS, MM group animals had 1.29 greater odds of being diagnosed pregnant compared to CON animals (Odd Ratio (OR) 1.29 95% C.I. 1.05-1.59). Interactions between farm and treatment and parity and treatment were far from significant (p=0.99 and 0.94), and therefore were removed from the model. When the model was calculated for animals with BCS<3 the MM group had an OR for a positive diagnosis of 1.19 compared to CON, but the difference was not significant (95% C.I. 0.93-1.53). Conversely, in animals with BCS≥3 OR for the MM group was 1.49 (95% C.I. 1.02-2.17) compared to CON.

Conclusions

The data shows, in line with previous literature, that a combination trace mineral injection applied at the beginning of the synchronization protocol can be beneficial for fertility in beef cows. Moreover, the magnitude of the benefit can be amplified in cows with adequate BCS, where energy might not be a limiting factor for reproduction.

1033 - Effect of treatment with human chorionic gonadotrophin 2 days after estrus on progesterone concentrations and reproductive outcomes in high-producing lactating dairy cows

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Objectives

Low conception rates after artificial insemination (AI) in high-producing lactating dairy cows negatively impact profitability and sustainability in dairy farms. Suboptimal concentrations of circulating progesterone (P4) in the early postovulatory period have been associated with lower pregnancy per AI (P/AI). High-producing dairy cows exhibit lower concentrations of P4 compared to low-producing dairy cows and nulliparous heifers, due in part to higher steroid metabolism, and this is associated with lower conception rates. Our previous work has shown that a single administration of human chorionic gonadotrophin (hCG) on Day 2 post-estrus in beef heifers increased corpus luteum (CL) area and serum P4 concentration as early as six days after estrus compared with control heifers (Maillo et al., 2014, doi: 10.1071/RD12353). In addition, administration of hCG two days after fixed-time AI (FTAI) increased the concentrations of circulating P4 in lactating dairy cows under pasture-based conditions (Sánchez et al., 2018, doi: 10.3168/jds.2017-14058). The aim of this study was to evaluate the effect of hCG treatment on Day 2 post-estrus on circulating P4 concentrations and P/AI in high-producing lactating dairy cows in confinement systems.

Material and methods

To that end, 805 lactating Holstein Friesian dairy cows from 15 farms located in the south of Spain were enrolled on this study. The mean (± SD) parity and days in milk (DIM) at enrolment were 2.3 ± 1.4 and 86.7 ± 17.8, respectively. After a voluntary waiting period (50-60 days after calving) cows underwent FTAI following a routine estrous synchronization protocol (Double Ovsynch, G6G, or 7-day Ovsynch + P4). Cows on each farm were blocked on parity, DIM, body condition score (BCS), and synchronization protocol and were randomly assigned to receive either 3000 IU of hCG (n=425) or an equivalent volume of saline solution (Control; n=380) on Day 2 after estimated estrus onset (~16 hours before FTAI=Day 0). Blood samples were collected by coccygeal venipuncture from a subset of 130 cows from both treatments (Control n=65 and hCG n=65) on Day 0, 7, and 14 of the estrous cycle to measure serum P4 concentrations. Pregnancy diagnosis was performed approximately 30 and 70 days after AI by transrectal ultrasonography. Statistical analyses were performed using SAS computational software. The effect of the independent variables on all binary response variables was determined using a generalized linear mixed model (GLIMMIX). Fixed effects in the model included treatment (Control or hCG), farm (1 to 15), BCS (≤2.50, 2.75 to 3.25, and >3.25), parity (≤2nd lactation, and ≥3rd lactation), DIM (≤80 d, and >80 d), and synchronization protocol (Double Ovsynch, G6G, or 7-days Ovsynch + P4). A logit link function was used and a binary response distribution was specified. For P4 analyses, once data were normally distributed and there was homoscedasticity, two-way ANOVA was performed.

Results

The increase in serum P4 concentration tended to be greater in cows treated with hCG compared with Control cows from Day 0 to 7 (14.8 *vs*. 11.3-fold increase, respectively; P=0.08) and from Day 0 to 14 (29.7 *vs*. 21.5-fold increase, respectively; P=0.06). No differences in the increase of serum P4 concentration were observed from Day 7 to 14 between groups. Only in ≥3rd lactation cows, those treated with hCG tended to have a greater P4 concentration compared with Control cows on Day 7 (3.68 *vs*. 2.88 ng/mL, respectively; P=0.10). P/AI on Day 30 after FTAI was affected by hCG treatment, parity, and farm. Moreover, there was a tendency for an interaction between treatment and parity. Overall, treatment with hCG 2 days after estrus increased P/AI (45.2% *vs*. Control 38.8%; P<0.05). In ≤2nd lactation cows, P/AI was similar between hCG-treated cows compared to Control (47.1% *vs*. Control 45.9%; P>0.05). Conversely, ≥3rd lactation cows treated with hCG had greater P/AI (42.1% *vs*. Control 27.3%; P=0.05). The overall incidence of embryo loss between Day 30 and Day 70 was 14.7%, and was not affected by treatment.

Conclusions

In conclusion, administration of hCG 2 days post-estrus increased fertility in ≥3rd lactation high-producing lactating dairy cows and this could be explained by an early increase in circulating P4. *Research projects RYC2021-033574-I and TED2021-129764B-I00 funded by MCIN/AEI /10.13039/501100011033 and by European Union Next GenerationEU/ PRTR*

1057 - Individual variation of circulating progesterone concentrations in Bos indicus cows receiving exogenous progesterone treatments

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Objetives

The objective of this study was to evaluate the individual variation of circulating progesterone (P4) concentrations in *Bos indicus* (Nelore) cows receiving intravaginal or intramuscular P4 treatments.

Material y methods

The data used in this study was obtained from two previous studies, in which Nelore cows were treated with intravaginal P4 devices (IVD) or long-acting injectable P4 (P4i). In the IVD study, 12 non-lactating Nelore cows were used (5.5 ± 1.8 years old; body condition score = 3.2 ± 0.1, 1 to 5 scale). In this study, cows were synchronized to have no corpus luteum (CL) at the time of the IVD (Sincrogest 1 g; Ourofino Saúde Animal) insertion, which was kept for 14 days. In the P4i study, 14 primiparous Nelore cows were used (36 ± 1.4 days post-partum; body condition score = 2.8 ± 0.1). All these cows had no CL when were treated im with 150 mg of P4i, (Sincrogest injetável, Ourofino Saúde Animal). In both studies, circulating P4 concentrations were determined by chemiluminescence assays. Data from daily blood collections until 14 days after treatments were analyzed separately for each study using Microsoft Excel software. The following measures for circulating P4 concentrations were analyzed per day and overtime: mean (), obtained by the function AVERAGE; standard deviation (*s*), obtained by the function STDEV.S, coefficient of variation (CV), as the ratio of the standard deviation to the mean; maximum and minimum values and range (R), obtained by the difference between the maximum and the minimum values.

Results

In the IVD study, the mean circulating P4 concentration overtime (from Days 1 to 14) was 1.72 ng/mL, with a CV of 33.7%. The minimum mean overtime observed was 1.10 ng/mL and the maximum mean overtime observed was 2.95 ng/mL. When the P4 concentrations were analyzed within each day, the highest CV (53.9%) was observed on Day 11, when the minimum P4 concentration observed was 0.59 ng/mL, and the maximum was 2.88 ng/mL. The lowest CV (20.5%) was observed on Day 7, when the minimum P4 concentration was 1.07 ng/mL, and the maximum was 2.10 ng/ml. In addition, on Day 1 the overall highest P4 concentration was observed, reaching a peak of 4.90 ng/mL, while on Day 13 the overall lowest P4 concentration was observed, reaching 0.44 ng/mL. In the P4i study, the mean circulating P4 concentration overtime (from Days 1 to 14) was 0.82 ng/mL, with a CV of 97.6%. The minimum mean overtime observed was 0.21 ng/mL and the maximum mean overtime observed was 2.99 ng/mL. When the P4 concentrations were analyzed within each day, the highest CV (109.2%) was observed on Day 12, when the minimum P4 concentration observed was 0 ng/mL, and the maximum was 0.72 ng/mL. The lowest CV (33.6%) was observed on Day 4, when the minimum P4 concentration was 0.40 ng/mL, and the maximum was 1.32 ng/mL. In addition, on Day 1 the overall highest P4 concentration was observed, reaching a peak of 6.38 ng/mL. Overall, the lowest P4 concentration was 0 ng/mL, which was observed in several cows from Day 6.

Conclusions

In conclusion, the analysis of the dispersion measurements for both studies demonstrated a considerable individual variation in circulating P4 concentrations among homogeneous groups of Nelore cows receiving the same P4 treatment, either by intravaginal or intramuscular administration. This study contributes to a better understanding of the pharmacokinetics of P4 supplementation in cows by distinct routes of administration, which are widely used in reproductive management strategies.

1068 - Investigating the metabolic signature of injectable multimineral based on organic and inorganic phosphorus, selenium, copper, magnesium, and potassium in beef cows

Author: Giovani Pastre Batista, Edson Lo Turco, Bruna Lima Chechim Catussi, Luc Durel, Bruno Sivieri de Lima, Pietro Sampaio Baruselli

Objectives
This study aimed to evaluate the metabolic profile and identify significant changes in metabolic pathways in response to the treatment with an injectable multimineral supplement containing organic and inorganic phosphorus, selenium, copper, magnesium, and potassium in primiparous beef cows. The experiment was conducted on a beef farm in Agua Clara, Mato Grosso do Sul state, Brazil.
Material and methods
A total of 50 Nelore (*Bos indicus*) primiparous cows, with body condition score of 3.05 ± 0,34 were randomly assigned to one of two treatments on D0 of fixed timed artificial insemination (FTAI) protocol: 1) Control (no treatment; n=25) or 2) Group 15mL (treated with 15mL of Fosfosal® on D0; n=25). The primiparous Nelore cows received a protocol based on D0 (average 40 days after calving) with 2 mg of estradiol benzoate and intravaginal progesterone (P4) device. On D8 0.533 mg cloprostenol (PGF), 1 mg of estradiol cypionate (EC) and 300 IU eCG were given concomitantly with P4 withdrawal. On D10 TAI was performed. Blood samples were collected for metabolomics analysis on D0 (beginning of FTAI protocol), D10, D21, and D39. Metabolites were evaluated using targeted LC-MS/MS analysis in tandem with a triple quadrupole mass spectrometer. Multivariate analysis as partial least squares discriminant analysis (PLS-DA) were performed by MetaboAnalyst 5.0. The top significant features were selected for Over Representation Analysis.
Results
A relative concentration of 145 metabolites was used to identify altered metabolic pathways following the multimineral treatment. On D0, no differences were observed between groups. However, on D10, metabolic pathways were impacted by treatment with 15mL of fosfosal on D10, which were tryptophan metabolism and spermine and spermidine biosynthesis (p=0,08/Q2-). On D21, changes were observed in the metabolism of ketone bodies, spermidine and spermine biosynthesis, butyrate metabolism, and mitochondrial electron transport chain (p=0,10/Q2+). On D39, alterations were observed in the mitochondrial electron transport chain and beta-alanine metabolism (p=0,10/Q2+). Following are the metabolic pathways induced by the treatment. **Tryptophan Metabolism:** The observed deviations underscore the extensive influence of the multimineral treatment on essential amino acid pathways. Tryptophan, a precursor for pivotal molecules like serotonin and melatonin, modulates cattle behavior and physiological functions. Also influences niacin (Vit B3) synthesis, immune function, and inflammatory functions and improves animal welfare. **Spermidine and Spermine Biosynthesis:** These pathways reveal the impact on polyamine metabolism, governing essential roles in cellular processes (cell growth, proliferation, and differentiation). Also, presents antioxidant properties (stress adaptation responses) and modulates sperm function and embryonic development, and improves animal welfare. **Ketone Body Metabolism:** Ketone bodies (acetoacetate, beta-hydroxybutyrate,

acetone) are produced during increased fatty acid breakdown periods. They reflect energy dynamics and nutrient utilization shifts, serve as alternative energy sources, particularly for the brain and muscles, during periods of reduced glucose availability. **Butyrate Metabolism:** Perturbations in this pathway might impact gut integrity and overall metabolic health. Short-chain fatty acid (SCFA) production during the microbial fermentation of dietary fiber in the rumen is related to energy sources and the maintenance of rumen epithelium acidification, adjusting the rumen environment. **Mitochondrial Electron Transport Chain:** Central to cellular respiration, this metabolic pathway influences ATPATP production through oxidative phosphorylation, impacting energy production and cellular functions. **Beta-Alanine metabolism:** Critical in linking carbohydrate and amino acid metabolism, it increases energy stores and ATP production, contributing to carnosine synthesis that reduces muscle acidosis, regulates muscle concentration and glycolysis, and impacts fatigue resistance.

Conclusions

In conclusion, on day 10, these pathways reflect alterations in amino acid metabolism, energy production, and lipid utilization. The persistent presence of spermidine and spermine biosynthesis underscores their role in modulating cellular growth and stress response. On day 21, changes hint at energy dynamics, gut health, and mitochondrial function. On day 39, metabolic pathways further emphasize the evolving impact of Fosfosal, reflecting alterations in lipid metabolism and energy production, expanding our comprehension of Fosfosal's metabolic footprint.

1069 - Pregnancy rates following timed-AI in lactating dairy cows synchronized with a Double OvSynch protocol using two different GnRH analogs.

Author: Gabriel BÓ, Paul RENAUD, Stefano ALLODI, Corinne MORSIANI

Objectives

To compare the efficacy of two different GnRH analogs in inducing ovulation and pregnancy rates following timed-AI (TAI) in lactating dairy cows synchronized with a Double OvSynch protocol.

Material and methods

The experiment was conducted on a commercial dairy farm near Mantova, Italy. The animals used were lactating Holstein cows (n=254) that were producing 30 to 60 L of milk per day, 40-50 days in milk, 1 to 7 lactations and with body condition scores $\geq$ 2.75 (1 to 5 scale), managed in a confinement system and receiving their first postpartum service. The experiment was a blind study in which all cows were randomly allocated to one of two treatment groups, to receive one of two different GnRH analogs in a Double OvSynch TAI protocol. Cows in Group 1 received Oestracton® (gonadorelin [6-D-Phe] acetate 52.4 µg/ml, Vetoquinol, Lure, France) in a 1 mL intramuscular (i.m.) injection, while those in Group 2 received Veterelin® (Buserelin acetate, 0.004 mg/ml, Calier, Spain) in a 2.5 mL i.m. injection. On Day -17 (day of initiation of treatments), all cows without records of clinical signs of illness (including reproductive diseases e.g., postpartum metritis, retained placenta, mucopurulent vaginal discharge, endometritis or ovarian cysts) were evaluated by ultrasonography and those without apparent reproductive abnormalities and with a CL and at least one follicle >10 mm and <25 mm in diameter received one of the two GnRH analogs. On Day -10, cows received a 2 mL i.m. of prostaglandin F2α (PGF2α, dl-cloprostenol sodium, 250 µg/ml, Bioestrovet®, Vetoquinol) and on Day -7 a second injection of the same GnRH analog. On Day 0, all cows received their respective GnRH analog, which was followed by PGF2α on Day 7 and another GnRH treatment with the same analog 56 h later (Day 9). On Day 10 (16 h after GnRH), all cows were TAI using semen of bulls with proven fertility. Furthermore, 118 cows in Group 1 and 122 cows in Group 2 were examined by ultrasonography on Days 0 and 7, to record the presence of CL/s to determine the efficacy of each GnRH analog in inducing ovulation and diameters of the two largest follicles. Cows were again examined by ultrasonography 30 and 60 days after TAI for pregnancy diagnosis. Data were analyzed using the GLM mixed procedure for binary data with a logit link (InfoStat, 2021) to determine the proportion of cows that ovulated after the treatment with each GnRH analog on Day 0 and pregnancy per AI (P/AI).

Results

The proportion of cows that ovulated after the first GnRH treatment did not differ (P=0.2) between groups (Group 1: 78.8% (93/118) and Group 2: 85.3% (104/122). Furthermore, 30- and 60-day P/AI did not differ between groups (49.2%; 62/126 and 42.9%; 54/126 vs 51.5%; 66/128 and 47.7%; 61/128, for Groups 1 and 2, respectively.

Conclusions

The P/AI obtained in this study (49.2% in Group 1 and 51.6% in Group 2), are within expectations when using Double OvSynch protocols in dairy cows. Furthermore, the

results of this study indicate that both GnRH analogs were efficacious in a Double OvSynch TAI protocol for high producing dairy cows. Finally, the results of this study also indicate that 500 µg of dl-cloprostenol sodium (Bioestrovet®, Vetoquinol) was an efficacious component in the Double OvSynch TAI protocol, regardless of the GnRH used.

1088 - Epidemiological survey of Leptospira borgpetersenii serovar hardjo type hardjobovis, serovar hardjo type hardjoprajitno, and L. interrogans serovar pomona in cattle in Colombia (2018-2023)

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Objectives

Infection due to bacteria of the *Leptospira* genus is a substantial condition that affects cattle reproductive performances globally. Bovine leptospirosis (BL) results in economic losses through abortions, infertility, stillbirth, and retained placenta by infecting the reproductive tract of cows. Researchers acknowledge that serovar *hardjo* is the leading cause of BL, which has been identified as two species, *L. interrogans* serovar *hardjo* (*hardjoprajitno* type) and *L. borgpetersenii* serovar *hardjo* (*hardjobovis* type), the latter being the most prevalent worldwide. However, due to the lack of national reports, the types of *Leptospira* serovars circulating in Colombian herds are mainly unknown. Although vaccination against reproductive diseases has increased recently, no significant advances have been observed. Researchers point out that not all vaccines include the *harjobovis* serovar in their composition. This study aimed to investigate the prevalence of *L. borgpetersenii* serovar *hardjobovis*, serovar *hardjoprajitno*, and *L. interrogans* serovar *pomona* in bovine serum collected from farms with a history of reproductive issues.

Material and methods

Over five years (September 2018-September 2023), bovine serum samples from different regions of Colombia were analyzed at the LABORATORIO MEDICO VETERINARIO S.A.S. (L.M.V.), Bogotá, Colombia. The blood samples used in the study were collected from cattle farms that were experiencing reproductive issues. VIRBAC Colombia (Bogotá) deployed four livestock field technicians operating the technical support program for cattle breeders in different regions to collect the samples. These regions include the leading dairy area (Sabana de Bogotá, Cordon Lechero de Antioquia, Boyaca, and Nariño), beef cow regions (Cesar, Cordoba, Magdalena Medio Llanos Orientales, Santander, Bajo Cauca), and other areas (Eje Cafetero, Cauca Valle del Cauca, Tolima). Serum were tested for *Leptospira borgpetersenii* serovar *hardjobovis* (n=5.212), serovar *hardjoprajitno* (n=4.505) and *L. interrogans* serovar *pomona* (n=3.715). All samples were analyzed using the microscopic agglutination test (M.A.T.) to detect anti-leptospira agglutinins, a standard test recommended by the World Organization for Animal Health (OIE). Animals were deemed positive when the antibody titer was 1:100 or greater.

Results

Out of 5.212 samples tested for *L. borgpetersenii* serovar *hardjobovis*, 3.279 (62.8%) were positive. For *L. borgpetersenii* serovar *hardjoprajitno*, 2.978 samples out of 4.505 tested positive (66.1%), and 2.092 samples out of 3.715 tested positive (56.3%) for *L. Interrogans* serovar *pomona*. No spatiotemporal analysis was conducted, but the national nature of the sample collection suggests that the three serovars are widely spread all over Colombia, significantly contribute to BL, and persist as enzootic infection.

Conclusions

This multi-year study in Colombia showed the significant prevalence of serovars *hardjobovis* and *hardjoprajitno* in the blood serum of cows experiencing reproductive disorders far beyond the seroprevalence reported by other investigators in randomly selected unvaccinated herds, with all serotypes combined.

Previous studies have shown that serovar *hardjobovis* is a highly prevalent serovar in cattle farming in Colombia. With the results of this study, we can confirm this premise, but always taking into account the high prevalence of serovar *hardjo* type *hardjoprajitno*, and *L. Interrogans* serovar *pomona*. Identifying the serovars that cause outbreaks on each farm is key for controlling bovine leptospirosis. This process can be quite a challenge, but it allows the development of a vaccination program adapted to each farm to combat the disease effectively.

Results gained in this survey emphasize the critical need for serovars *hardjo* type *hardjobovis* and type *hardjoprajitno* as well as *L. Interrogans* serovar *pomona* in the formula of vaccines considered for large-scale strategic vaccination programs. Unfortunately, not all reproductive vaccines have serovars *hardjo* type *hardjobovis* and type *hardjoprajitno* in their composition, which limits the ability to control these serovars effectively since the vaccines do not confer cross-immunity between the different serovars.

1093 - Mineral supplementation by injection during the synchronization protocol increases the size of the dominant follicle of the ovary and the conception rate

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Objectives

Fixed-time artificial insemination (FTAI) is a well-known method of conducting reproduction in cattle. The protocols are safe and now well-controlled. However, their performance may fluctuate due to animal factors or the suitability of the cow for this treatment. Marginal deficiencies in minerals such as phosphorus (P), potassium (K), magnesium (Mg), copper (Cu), and selenium (Se) have been reported to cap reproductive efficiency and calf production in beefcow herds. Therefore, strategic mineral supplementation beyond the requirements (top-up) at the start of the FTAI process could help improve reproductive performances. The first objective of this study was to assess the impact of injecting minerals at the initiation of the reproduction protocol on the conception rate and pregnancy success. The second objective was to evaluate the effects ofinjecting minerals once versus twice during an FTAI program on ovarian activity.

Material and methods

Two experiments were conducted on a beef cattle operation in the Mexicali Valley in Northern Mexico. Three hundred multiparous Angus cows were enrolled in this study. Animals were subject to a FTAI program that included the application of a progesterone-releasing intravaginal device from D0 to D7 (1 g progesterone) plus an injection (i.m) of estradiol benzoate (2mg) on D0, followed by i.m. injections of cloprostenol (0.150 mg), estradiol cypionate (0.5 mg), and equine chorionic gonadotropin (400 UI) on D7. On D7, the intravaginal device was removed, and FTAI was performed 48 hours later. Meanwhile, on D0, a subset ofthe experimental population (n=172, MIN group) was dosed with 10 mL, i.m. of a multimineral solution providing to each cow 1.4 g of sodium glycerophosphate, 2.01 g of monosodium phosphate, 40 mg of copper chloride, 60 mg of potassium chloride, 250 mg of magnesium chloride, and 24 mg of sodium selenite (FOSFOSAN®, Virbac Mexico, Zapopan, Jal.). One hundred and nine cows (109) were not supplemented (CTRL group). Another subset of the population (n=32, MIN-1) was dosed on D0 with the mineral solution (10 mL, i.m.), while the last group (n=32, MIN-2) was dosed twice onD0 and D7 (same dosage). The estrus was identified in the presence of cervical mucus during the FTAI protocol. A pregnancy check was performed at 39 days postinsemination. This information was used to calculate the estrus rate (cows in estrus/cows treated) and the conception rate (pregnant cows/cows inseminated). In the MIN-1 and MIN-2 groups, the ovarian activity was determined by scanning ovaries on D0, 1, 3, 5, and 7 of the program. Emerging follicles were identified and monitored until they reached dominance or atresia. The endpoints of this work were follicular wave emergence (FWE: time elapsed between D0 and the onset of the follicular wave), size of emerging follicles (average diameter of all detected follicles ≥2 mm), and dominant follicle size (average diameter of the largest follicle detected on D7).

Results

Both the conception rate (55.05 *vs.* 66.86 %, for CTRL and MIN, respectively) and estrus rate (44.04 *vs.* 55.81 %, for CTRL and MIN, respectively) significantly increased ($P<0.05$). While the FWE appeared 0.5 days earlier ($P<0.01$), the dominant follicle was 1.62 mm smaller ($P=0.01$) using two injections than one. There was no effect ($P\geq0.12$) of treatment in therest of the variables associated with ovarian activity.

Conclusions

While two mineral injections seven days apart produced an early follicular wave emergence, these results established that a single injection of 10 mL of the detailed mineral solution at the start of an FTAI program was enough to improve the reproductive parameters of lactating Angus cows.

1126 - Vaccination with Brucella abortus strain RB51 at the beginning of E2/P4-based TAI protocol does not affect pregnancy and calving rates of healthy Bos indicus beef cows

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Objectives

Bovine brucellosis is a zoonosis that has been a target in eradication programs in several countries due to concerns on public health and its negative impact on livestock production. Besides abortion, *Brucella abortus* causes birth of weak offsprings and low fertility. One of the main alternatives for eradication is the vaccination with *Brucella abortus* strain, which is recommended by the World Organization for Animal Health. Therefore, the hypotheses for this study was that treatment with *Brucella abortus* strain RB51 [Bovilis RB-51®, MSD Animal Health, São Paulo, Brazil] at the beginning of Estradiol/Progesterone (E2/P4)-based TAI (Timed Artificial Insemination) protocol does not affect healthy female fertility and demonstrates that the vaccine can be used prior TAI in Brazilian commercial farms as part of a strategy to control brucellosis.

Material and methods

Therefore, Cia Pecuária Company at Rancho Alegre Ranch conducted a study, in Miranda, Mato Grosso do Sul, Brazil, from October 2016 to January 2017. A total of 724 Nelore and crossbred females from two commercial farms, including heifers (n=219; $BCS_{median|Stderr}$=3.00±0.02; min=2.50; max=3.50), primiparous (n=59; $BCS_{median|Stderr}$=2.50±0.03; min=2.00; max=3.00) and multiparous (n=446; $BCS_{median|Stderr}$=2,50±0.02; min=1.75; max=3.50) were randomly assigned to a treatment with 2 mL dose of a vaccine with *Brucella abortus* strain RB51 (n=349; RB51) or Control (saline solution, n=375) group. Treatment was performed subcutaneously on the first day of E2/P4-based TAI protocol (Day 0). Pregnancy diagnosis was carried out 30 days after TAI and all calvings were registered. Descriptive statistics and inference were performed using SAS/STAT software, included Freq, Means and Glimmix procedures. Category (heifer, primiparous or multiparous), breed, farm, sire semen, AI technician, heat and body condition score were recorded and included in the model as random effects.

Results

No interactions between treatment and other studied variables were found. Confirming our initial hypothesis, P/AI between treated and control groups were similar [Saline = 64.3% (241/375) and RB51 = 65.3% (228/349); P=0.70] and also the calving rate [Saline = 89.3% (334/374) and RB51 = 89.7% (312/348); P=0.96]. However, secondary effects for P/AI were observed on category [primiparous = 76.3% (45/59)^{a}, multiparous = 66.8% (298/446)^{ab,} Heifers = 57.5% (126/219)^{b}; P=0.01]; sire semen [A = 74.9% (173/231)^{a}, B = 61.7% (166/296)^{b}, C = 58.0% (130/224)^{b}; P=0.006] and statistical trend for farm [A = 67.0% (417/693)^{a}; B = 51.5% (52/101)^{b}; P=0.07].

Conclusions

In conclusion, the vaccination with *Brucella abortus* strain RB51 vaccine can be performed on the first day of E2/P4-based TAI protocol with no negative effects on pregnancy rate and calving rate of Nelore healthy cows. Therefore, the vaccine can be an important strategy to support farms aiming to eradicate brucellosis.

1135 - Association between calving season (warm versus cold) and fertility of dairy cows in the temperate region of Mexico

Author: Ulises Esau Medina, Joel Hernandez Ceron, Jorge Antonio Ruiz, Noé Juárez

Objectives
The objective of this study was to compare the fertility of dairy cows calved in two different seasons of the year in the central highlands of Mexico.
Material and methods
This is a retrospective observational study in which reproductive and productive records of a total of 2114 lactating Holstein cows (366 primiparous and 1748 multiparous) from a dairy herd located in Querétaro, Mexico (cows were included if they calved between January 2021 and April 2022) were analyzed. Cows were housed in a freestall barn with sand beds, shade and natural ventilation. Cows were milked thrice daily, eight hours apart. The feed delivered was formulated to meet the dietary requirements established for dairy cows NRC (National Research Council).
Health, production and reproduction data were retrieved from the herd management software. Based on the herd average predicted 305-d milk yield (11600 kg), cows were categorized into two lactation yield levels: high or low. Throughout the study period, 48 reports per day (for each 30-min period) of both ambient temperature and relative humidity data were used to calculate the Temperature-Humidity Index (THI). Derived from previous researches about heat stress, and for the purposes of this study, the stress threshold was considered from a THI of 68 units. The daily and monthly total hours in which cows were exposed to a THI ≥ 68 (heat load duration) were estimated. The monthly average heat load of the total study period (185 hours month-1) was used to define the calving season, considering months with a monthly heat load less than 185 hours (November-February) as the cold season, while months exceeding this average (March-October) were considered the warm season. Data were analyzed using multivariable logistic regression models to determine the effects of calving season, postpartum health, and milk yield on pregnancy success. Additionally, the Kaplan-Meier methodology was developed for graphical presentation of the survival of the calving season variable over time (interval from calving to conception).
Results
Cows that calved during the warm season had lesser odds of pregnancy at first postpartum artificial insemination (AI) that those cows calved during the cold season [43.7% (485/1108) vs. 49.8% (501/1006); OR: 0.70; 0.61-0.87; $P < 0.05$]. Cows with low lactation yield had greater odds of pregnancy compared with high lactation yield [50.7% (537/1059) vs. 42.5% (449/1055); OR: 1.5; 1.24-1.76; $P < 0.05$]. The pregnancy was most likely in cows clinically healthy compared with cows diagnosed with at least one clinical disease during postpartum period [50.2% (782/1555) vs. 36.4% (204/559); OR: 1.8; 1.36-2.12; $P < 0.05$]. The interaction between calving season and lactation yield was nonsignificant ($P > 0.05$). The median days in milk until pregnancy were 170 (159-184) and 132 (124-141) for cows calved during the warm season and cold season, respectively (median; 95% CI; $P < 0.05$).
Conclusions
Our results suggest that calving season influences on the fertility of dairy cows in the central highlands of Mexico. Cows calved during the warm season had lower probability

to become pregnant at the first postpartum AI and had longer time to conception (open days) than those cows calved during the cold season. Until now, the effect of heat stress has not been considered in the strategic management of dairy herds in the region with the aim of improving reproductive performance.

1161 - Epidemiological study of the main infectious abortifacient diseases in Morocco.

Author: Zaid Zouagui, Yassine Bougtab, Achraf Boukili-Idrissi, Juan Munoz-Bielsa, Carla Azevedo, Philippe Gisbert, Jaouad Berrada

Objectives

In cattle, abortions deserve particular attention because of their heavy economic and hygienic impact.

The aim of the present work is to carry out an epidemiological study of abortion frequencies in dairy cows during the 2022-2023 agricultural season in Morocco's main dairy basins, namely the Loukkos, Gharb, Fès, Chaouia, Doukkala, Tadla, Souss Massa and Laâyoune regions.

Material and methods

The epidemiological survey covered 77 farms with a total of 33,463 head of adult female cattle. The study also included serological investigations into the main infectious abortifacient diseases, particularly brucellosis (*Brucella sp.*), Q fever (*Coxiella burnetii*), leptospirosis (*Leptospira*hardjo), salmonellosis (*Salmonella* dublin), neosporosis (*Neospora caninum*), and bovine Herpes virus 4 infection.

The serological survey covered a total of 535 randomly selected adult females of dairy breeds of different ages. Serological screening for brucellosis was carried out using the Rose Bengal Test, while serological screening for the other five infections was carried out using an indirect ELISA test. For Q fever, in addition to serological analysis, biomolecular PCR screening was carried out on bulk tank milk (BTM). This involved 72 samples of BTM from 57 farms and 11 collection centers representing between 18 and 43 small farmers with a total of 1600 lactating cows. Four large farms had two tanks.

Results

The survey data revealed an overall abortion frequency of 14.2% for the 2022-2023 year. Abortion frequencies varied from region to region, ranging from 2.1% in the Fez region to 17.6% in the Loukkos and Gharb region. The average intra-farm abortion rate was 5.4%.

Serological analyses showed that all the abortifacient agents investigated were present in the 7 regions where investigations were carried out. BoHV-4, Neosporosis, Q fever and leptospirosis infection showed seroprevalence levels of 59%, 56%, 31% and 27% respectively. Overall seroprevalence of brucellosis was 3.4%, particularly in the Loukkos, Gharb, Chaouia and Doukkala regions. The overall seroprevalence of salmonellosis at animal level was 3.6%, with the absence of infection in the Fes and Laâyoune regions. Neosporosis, BoHV-4, Q fever, leptospirosis, salmonellosis and Brucellosis were found in 91%, 81%, 63%, 44%, 13% and 7,8% of farms respectively. At herd level, prevalences were 55% for BoHV-4, 48% for neosporosis, 24% for Q fever, 16% for leptospirosis, 7.8% for brucellosis and 3% for salmonellosis.

Among cows aborted in the 2022-2023 campaign, seroprevalence rates were 59% for BoHV-4, 44% for neosporosis, 22% for Q fever, 15% for leptospirosis, 7% for salmonellosis and 3.7% for brucellosis. It should be noted that 22% of cows were found to be seronegative for all infectious agents.

Biomolecular analysis of BTM revealed that 49% of samples were positive for the Q fever agent. Only one collection center out of 11 was positive. This shows that the bacterium is actively circulating in almost one herd in two mostly in medium and large dairy farms. Furthermore, 36% of ELISA-positive farms were PCR-negative, and only one ELISA-negative farm was PCR-positive.

Conclusions

Based on the results of this study, it is recommended that a bovine abortion control program should be implemented in Morocco, based on health surveillance, accurate laboratory diagnosis and medical prophylaxis.

1184 - Reproductive efficiency of two fixed-time synchronization protocols: Ovsynch and the use of a Controlled internal drug release plus equine chorionic gonadotropin (eCG) in lactating dairy cows.

Author: Gabriela Mapes, Marion Galindo, Carlos Corona

Objectives

OBJECTIVE: To compare the pregnancy rate in high-producing dairy cows under two different reproductive management strategies for fixed-time insemination (IATF): Ovsynch and the use of a Controlled internal drug release (CIDR® 1900 Cattle Insert) plus equine chorionic gonadotropin (eCG).

Material and methods

MATERIAL AND METHODS: The study was carried out in the Torreon Region (25° 44' N, 103° 10' W at 1,111 masl). Holstein cows of different calving numbers were used with an average production of 11,000 kg of milk per lactation. Cows (n=700) were presynchronized with two injections of PGF2α at 35 and 47 days postpartum and on day 60 postpartum they were randomly assigned to two treatments (n=350): 1) The OV group received the Ovsynch protocol: 100 µg of GnRH (i.m.; day 1), 25 mg of PGF2α (i.m.; day 7) and 100 µg of GnRH (i.m.; day 9) and 16 hours after GnRH, the Fixed Time Artificial Insemination (IATF); 2) The controlled internal drug release + eCG group received the protocol of 1.9 g of Progesterone (CIDR®), the intravaginal device was placed and 2 mg of Estradiol Benzoate (BE) was administered (i.m.; day 1), controlled internal drug release withdrawal and 25 mg PGF2α (Lutalyse®) (i.m.), 400 IU eCG (Novormon 5000®) (i.m) and 1 mg Estradiol Cypionate (ECP®) (i.m.; day 7) were administered and 56 hours after removal of the controlled internal drug release, IATF was performed. The diagnosis of pregnancy was made 32 days after insemination with ultrasound and the pregnancy was subsequently reconfirmed at 60 days by rectal palpation.

Results

RESULTS: The pregnancy rate was evaluated for both groups, with a significant difference between the groups (OV=42%, The controlled internal drug release + eCG =67%; P<0.01). The odds of not becoming pregnant at the end of the IATF were 1.8 (95% CI=1.839-2.426) times higher for the Ovsynch group than for the controlled internal drug release + eCG group. Body Condition was evaluated at day 60 postpartum, day 32 and day 60 post insemination. Differences in pregnancy rates were observed in those cows that lost CC (<3.5) during the study, showing a greater tendency to become pregnant with the controlled internal drug release + eCG (43% vs 33% Ovsynch).

Conclusions

CONCLUSIONS: It is concluded that the protocol with the controlled internal drug release + eCG improved the pregnancy rate, consequently, it improved the reproductive efficiency and the economic return of the herd.

1228 - Veterinary practitioner-recorded bovine congenital defects using a new app.

Author: John Mee F

Objectives

Congenital defects may be defined as abnormalities of structure and function present at birth (1). The objective of the project was to field-test a mobile phone app designed for use by veterinary practitioners to collect clinical case data on congenital defects during routine farm visits.

Materials and methods

A 15-question questionnaire was designed, piloted with veterinary practitioners, and then formatted as a mobile phone app using Typeform (www.typeform.com). In addition, up to three photographic images or videos could be recorded per case. The app was provided to all veterinary practitioners (n=190) in an Irish practice group in December, 2020. Epidemiological information and images were collected during routine farm visits (mainly calvings). The questionnaire data and the images were stored in a secure azure cloud server and presented as an interrogatable dashboard using Microsoft Power Bi. In total, 191 congenital defect cases were categorised by the body system/s affected (2).

Results

In total, 59 vets in 28 XLVets Ireland veterinary practices distributed nationally participated in the project across three years (2020-2022). The number of cases/vet and /practice varied between 1 and 23 and 1 and 43, respectively.

The three most commonly recorded individual defects were intestinal atresia (24.1%), *Schistosomus reflexus* (20.4%) and ankylosis (6.8%); multiple defects were recorded in 13.1% of cases. Intestinal atresia included atresia of the intestines (43) and atresia ani (3). Multiple defects most commonly affected the musculoskeletal system (22/25 cases) and of these, those involving ankyloses (11) and palatoschisis (6) were the most common.

The majority of dams were pluriparae (141), of a dairy breed (138); the most common dam breed was Holstein-Friesian/cross (132). The majority of sires were of a beef breed (93), with the remainder dairy (81) and other. The most common sire breeds was Holstein-Friesian/cross (81) and the majority of cows were bred by artificial insemination (77). There was a similar number of male (43) and female cases (42) but the majority of cases had an unrecorded sex (106). The majority of cases were singletons (178) with the remainder twins (13). Gestation was full term in the majority of cases (180). The majority of cases were assisted at calving [Caesarean section (46), non-surgical assistance (41) or foetotomy (20)]. The majority of cases were recorded at birth (76) with the remainder in the first day (21).

Conclusions

This study highlighted two congenital defects of cattle which are often considered as sporadic in occurrence and of low prevalence. Based on the findings reported here, both intestinal atresia and *Schistosomus reflexus* need to be viewed in a new light as clinically important bovine congenital defects warranting preventive strategies at farm and national levels. The results of this study also indicate that veterinary practitioner

surveillance using mobile phone apps could be expanded to include other clinically important conditions and in other countries.

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1241 - Comparative study of fertility using an intravaginal progesterone insert DIB® or PROGESTAR®

Author: German Dominguez, Maximiliano Itterman, Gustave Decuadro Hansen, Rodolfo Luzbel De La Sota, Martin Salina, Julian Jaeschke

Objectives

In dairy cows, compare the effectiveness of the fertility obtained using an intravaginal progesterone (P_4) insert DIB® with 1.0 g of P_4 or PROGESTAR® insert with 0.96 g of P_4.

Material and methods

The study included 671 cows from 1 (LAC1) or 2+ lactation (LAC2+) cows diagnosed cycling or in superficial anestrus. The diagnosis was made by ultrasound (Tringa, Easote Piemedical) classifying, cycling any animal that had a CL, and in superficial anestrus, any animal that had an absence of CL and uterine tone and also a follicle >10 millimeters. All animals with odd ear tag numbers (n=356) were assigned to the DIB group, and on day 0, they were placed with a P_4 insert (DIB®; 1.0 g, Zoetis, Villa Adelina, BA) for 7 days, and all animals with even ear tag (n=315) were assigned to the PROGESTAR group and on day 0 a P_4 insert (PROGESTAR®; 0.96 g, Biogenesis-Bagó, SP, Brasil) was placed for 7 days. All animals were then administered 2 mg estradiol benzoate (BE; Bioestrogen®; Biogenesis-Bagó, Garín, BA). On day 7 of treatment, the insert was removed and both groups received 150 µg of D-cloprostenol (PGF; Enzaprost DC®, Biogenesis-Bagó, Garín, BA), 1 mg of estradiol cypionate (CE; Croni-CIP®, Biogenesis-Bagó, Garín, BA) and 400 IU of eCG (Ecegon®, Biogenesis-Bagó, Garín, BA).

Additionally, the base of the tail was painted (Fil Detail®; GEA, Düsseldorf, Germany), and the paint's score was read at 48 hours and 60 hours after insert removal. All animals with paint score reduced at 48 h were AI, and the rest were administered 10.5 µg of buserelin acetate (Gonaxal®; Biogenesis-Bagó, Garín, BA) and were FTAI at 60 h. Pregnancy diagnosis was made by ultrasound (Tringa, Easote Piemedical) at 28-42 d post FTAI.

Data were analyzed using a logistic regression model (SAS 9.4). The response variable studied was the rate of pregnancy to the FTAI. The independent variables include in the analysis were treatment (TRT; DIB vs. PROGESTAR), number of births (NPAR; 1 vs. 2+), number of services (NSER; 1 vs. 2+), cyclicity (CICLIC; superficial anestrus vs. cycling), body condition at the time of protocol initiation (BCC; <2.75 vs. ≥2.75), the bull (BULL; 1-7), the AI operator (INSEM; 1-3), and time of the FTAI (TIME; 48 vs. 60 h). Besides, days postpartum days (dpp) and milk production (MILKP, l/d) were included as covariates in the analysis.

Results

The cows assigned to the DIB or PROGESTAR group had similar number of lactations, milk production, and BCC at the time of treatment and at the time of AI (P>0.23). The cows of the PROGESTAR group had a lower number of days postpartum, and number of services (P 0.02), but only DPP affected the pregnancy rate. The percentage of cows cycling at the beginning of the protocol was similar between both groups (80.92%; P=0.98), and the pregnancy rate at AI was also similar between both groups (41.72% [141/338] vs. 46.25% [142/307], P=0.24). The pregnancy rate was similar among the cows AI at 48 or 60 h (43.91; P=0.81), among bulls (44.01%; P=0.87), and among AI

operators (43.88%; P=0.38). In the logistic regression analysis model, where all terms of the model were included, only DPPs had a significant effect on pregnancy rate (P=0.01).

Conclusions

Similar fertility was obtained in dairy cows using a DIB® insert with 1.0 g of P_4 or a PROGESTAR® insert with 0.96 g of P_4.

1243 - Study the activity of the eCG diluting it with saline solution or with D-Cloprostenol.

Author: Julian Jaeschke, Gustave Decuadro Hansen, German Dominguez, Martin Salina, Gustavo Muller, Esteban Galarza

Objectives

The objective of this study was to assess if the dilution of eCG in D-Cloprostenol would enable the administration of both drugs in a single injection, in Angus heifers, without losing effectiveness.

Material and methods

The study included 22 Angus Heifers of 12 month. The ultrasound were made with the equipment (Honda, HS1600) classifying all the heifers as prepuber (animal that never had a heat before). To all animals on day 0 they were placed with a progesterone intravaginal device (Cronipres; 0,5 g, Biogenesis-Bagó, Garín, BA) for 8 days and administered 2 mg of estradiol benzoate (BE; Bioestrogen; Biogenesis-Bagó, Garín, BA). At day 4 the eCG was administered (1500 UI, Ecegon; Biogenesis-Bagó, Garín, BA), the animals were randomly assigned to one of the three experimental groups; 7 animals for Traditional group (eCG was dilute with saline solution), 7 for Control group (without eCG) and 8 to Combined group (eCG was dilute with D-Cloprostenol). On day 8 of treatment, the intravaginal device was removed and the tree groups received 150 µg of D-cloprostenol (PGF; Enzaprost DC, Biogenesis-Bagó, Garín, BA), 1 mg of estradiol cypionate (CE; Croni-CIP, Biogenesis-Bagó, Garín, BA).

Ultrasounds were performed at different times of the trial; on day 0 to confirm all the heifers were prepuberal, also on day 8 to evaluate the number of follicles >8mm per animal, on day 10 to evaluate the number of follicles >10mm per animal and o day 17, to evaluate the number of corpus luteum (CL) per animal.

Data were analyzed with Kruskal-Wallis analysis (R programming environment; version 4.2.2; R Core Team, 2022). The response variable studied was the number of CL. The independent variable was treatment (Traditional vs Combined vs Control). The experimental unit in the study was the cow. Post hoc comparisons were performed using the Wilcoxon test with Holm adjustment implemented in R. This test was conducted using the pairwise.wilcox.test function from the stats package. The Holm adjustment was applied to correct for multiple comparisons and control the overall Type I error. Significance was set at a level of $\alpha = 0.05$.

Results

A treatment effect was observed (P<0,01). There was no different number of CL between Cows from Traditional or Combined Groups (11,3 vs 10,0, respectively; P=0,35), but these groups but these groups had a greater number of CL than the Control group (0,3; P<0,01).

Conclusions

Diluting the eCG with the D-Cloprostenol does not interfere with the activity and effectiveness of the glycoprotein.

1262 - Efficacy of a 6-day CO- Synch protocol in beef in herds in Pozoblanco region (southern Spain)

Author: Teresa Flores Barragan, Francisco Javier Martínez Blasco, Jose Ignacio Muñoz

Díaz, Miguel Jesús García Marín, Gabriel Moyano Ortega, Santiago Pérez Wallace, Eber Rojas Candas

Objectives

This study aimed to assess the efficacy of the 6-Day CO-Synch+ P4 protocol in cows and heifers from the Pozoblanco region, providing local data not previously reported. The investigation focused on ovulatory response after GnRH administration (GnRH-1) and initial corpus luteum presence and pregnancy rates (PR) after artificial insemination (IATF) in heifers and beef cows subjected to a modified 6-Day CO-Synch+ P4 protocol.

Material and methods

Limousine heifers (n=92, age: 20±2 months), Limousine cows (n=50), and other breeds of cows (n=16) and heifers (n=34) from 15 farms in southern Spain received a 1g progesterone intravaginal device (DIB®, Maymo, Spain) and 100 µg gonadorelin (Prolusyn®, Maymo Spain) at the protocol's initiation (Day -9). On Day -3, the P4 device was removed, and 1,000µg cloprostenol sodium (Syncroceliven®, Maymo, Spain) and 300 IU equine chorionic gonadotropin (eCG; Fixplan®, Maymo, Spain) were administered. Insemination and 100 µg gonadorelin followed 72 hours post-device removal. Ultrasound examinations at (Day -9) and (Day -3) assessed ovarian structures in a subset of cows (n=22) and heifers (n=68). The pregnancy rate was determined by ultrasonography 35 days after artificial insemination. All data collected were analysed with PROC LOGISTIC of SAS software (Statistical Analysis System, Version 9.4 for Windows; SAS Institute Inc., Cary, NC ®). Response variables were CL presence (cyclicity) on Day -9, Response to the GnRH on Day -9 and pregnancy rate. Explanatory variables were herd, category (cow/heifer), body condition score, and breed.

Results

Cyclicity rate at device insertion was 84.4% (29/186), unaffected by the farm (P=0.70) or category (P=0.67). Ovulation to the first GnRH was 28.9% (26/90), higher in animals with dominant follicles (>10 mm; 39.0% vs. 17.9%; P=0.04) and unaffected by breed, farm, category, or CL presence (P<0.1). The pregnancy rate was 60.7% (116/191). Cycling cows exhibited higher PR than non-cycling (65.3% vs. 34.6%; P=0.005), and PR was greater in animals responding to GnRH-1 (73.1% vs. 50.0%; P=0.049). PR was not affected by breed, farm, or category (P>0.1).

Conclusions

The presence of corpus luteum at protocol initiation increased fertility rates in both cows and heifers, as did an ovulatory response to the first GnRH (Day -9). In summary, the 6-Day CO-Synch+ P4 protocol proves practical and effective for application in beef herds in southern Spain.

1268 - Pregnancy per AI in grazing suckled beef cows subjected to estradiol salts- or GnRH-based fixed-time AI protocols

Author: Luis Ferré, Natalia Cerviño, Julian Jaeschke, Maximiliano Itterman, Jaime McLean, Agustin Aragon, Manuel Fernández, Matías Villa, Néstor Formia, Ramiro Rearte, Marcos Colazo, Jordan Thomas, Luzbel de la Sota, Gustave Decuadro-Hansen

Objectives

Fixed-time artificial insemination (FTAI) protocols can be generally divided into two groups: 1) estrogen salts (ES) + prostaglandin (PGF2α) + intravaginal progesterone device (DIV) and, 2) gonadotropin-releasing hormone (GnRH) + PGF2α + DIV. While both programs have been used successfully in several countries, the use of GnRH-based protocols has not been widely spread in Argentina. However, the use of ES in beef herds willing to export meat to the European Union is prohibited. Therefore, there is a need to compare the efficiency of the GnRH-based protocols versus ES-based protocols in grazing beef cattle. The objective of this study was to compare the effect of GnRH-based FTAI protocols versus a standard ES-based FTAI protocol on pregnancy per AI (P/AI) in grazing suckled beef cows.

Material and methods

Multiparous Angus cows (n=513) with at least 45 days postpartum with an average body condition score (BCS, scale 1-5) of 3.01±0.27 were enrolled in this study. Before FTAI protocol initiation, an ultrasound examination of the ovaries was done to determine the presence of a corpus luteum (CL) and the diameter of the largest follicle (FOL). Cows were classified as cycling (CY; with CL, n= 39%), superficial anestrous (SA; without CL, with FOL ≥10mm, n=38%), and deep anestrous (DA; without CL, with FOL ≤10 mm, n=23%). Cows were randomly allocated into one of four FTAI protocols considering BC and ovarian status. All cows received a DIV containing 0.5 g of P4 on the day of protocol initiation and administration of 150 µg PGF2α plus 400 IU of equine chorionic gonadotropin (eCG) at the time of device removal. The four FTAI protocols (PRO) were: 1) 7-Day ES+1XDIV (PRO1, n=129), 2 mg of estradiol benzoate (EB) at DIV insertion on day (d)-9 and 1 mg of estradiol cypionate (ECP) at DIV removal on d-2, and FTAI on d0; 2) 7-Day GnRH+1X DIV (PRO2, n = 129), 10 µg of GnRH at DIV insertion on d-10, DIV removal on d-3 and GnRH and FTAI at d0; 3) 7&7 GnRH+1X DIV (PRO3, n = 128), PGF2α at DIV insertion on d-17, GnRH on d-10, DIV removal on d-3 and GnRH and FTA at d0I; and 4) 7&7 GnRH+2X DIV (PRO4, n = 127), PGF2α at DIV insertion on d-17, GnRH on d-10 plus DIV replacement with a new device, DIV removal on d-3 and GnRH and FTAI at d0. Biogenesis-Bagó, Argentina, provided all pharmaceuticals used in the study. All cows were tail painted at DIV removal to identify those in estrus at FTAI (YES= ≥50% paint removed). Cows were inseminated by one technician using frozen-thawed semen from a sire at 50-52 or 64-68 h after DIV removal for ES- or GnRH-based FTAI protocols, respectively. Pregnancy was diagnosed by ultrasonography 35 d after FTAI. The effect of FTAI protocols on P/AI was assessed by fitting a logistic regression model with ovarian status and estrus expression as main effects and BCS as a covariate. The same model was also used to assess the interaction between estrus expression and FTAI protocols (PROC GLIMMIX, SAS, 9.4).

Results

Pregnancy per AI did not differ among PRO1, PRO2 and PRO3 (48.3, 46.5 and 53.2%; P=0.09). However, P/AI was greater for PRO4 compared to PRO1 (58.6 vs. 48.3%; P=0.05). Cows that expressed estrus had higher P/AI compared to cows that did not expressed estrus (58.9 [n=438] vs. 44.9% [n=75]; P=0.04). In cows that did not express estrus, P/AI did not differ among FTAI protocols; however, in those expressing estrus, P/AI was higher in the PRO4 compared to PRO1 and PRO2 (67.8% [n=127] vs. 54.7 [n=129], P=0.04; 67.8% [n=127] vs. 52.8 [n=129], P=0.02); respectively). The BCS at the time of initiation of FTAI protocol improved P/AI (P=0.009) whereas ovarian status did not (P=0.63) affect P/AI.

Conclusions

The standard GnRH-based FTAI protocols (PRO2 and PRO3) resulted in P/AI similar to that obtained with the standard ES-based protocol (PRO1). However, the use of a 7&7 GnRH+2X DIV (PRO4) improved P/AI by 15% compared to the standard protocols 7-Day ES+1XDIV (PRO1) and 7-Day GnRH+1X DIV (PRO2) particularly in those cows expressing estrus. MNC has a fellowship from INTA-CONICET. This study was funded by PICT-2021-I-A-00312 and by IPCVA-2023-29389 to LBF and RLS.

1269 - Pregnancy per AI in 22-month-old Angus heifers synchronized with either estradiol salts- or GnRH-based fixed-time AI protocols

Author: Luis Ferré, Natalia Cerviño, Julian Jaeschke, Maximiliano Itterman, Jaime McLean, Agustin Aragon, Manuel Fernández, Matías Villa, Néstor Formia, Ramiro Rearte, Marcos Colazo, Jordan Thomas, Luzbel de la Sota, Gustave Decuadro-Hansen, Carlos Montbrau

Objectives

In South America, fixed-time artificial insemination (FTAI) programs are generally based on estradiol salts (estradiol benzoate-BE and estradiol cypionate-ECP). While these programs have been successful, gonadotrophin releasing hormone (GnRH)-based protocols have been reported to be equally effective in other regions. The objective of this study was to compare pregnancy per AI (P/AI) in heifers synchronized using either estradiol salts (ES) *or* GnRH with or without a pre-synchronization scheme.

Material and methods

Angus heifers 22±2 months of age with 308.6±24.0 kg of weight and an average body condition score (BCS, on a scale of 1 to 5) of 2.82±0.11 were enrolled in this study. Before FTAI protocol initiation, an ultrasound examination of the ovaries was done to determine the presence of a corpus luteum (CL) and/or the diameter of the largest follicle (≥10mm). Heifers with a CL were considered cycling (CY), with no-CL + follicles ≥10mm in superficial anestrous (SA) and no-CL + ≤10 mm in deep anestrous (DA). Only CY and SA females were enrolled in the study. Heifers were randomly allocated into one of three FTAI protocols considering weight, BCS, and ovarian status. All heifers received an intravaginal device (DIV) containing 0.5 g of P4 on the day of FTAI protocol initiation and administration of 150 µg PGF2α plus 300 IU of equine chorionic gonadotropin (eCG) at the time of DIV removal. The three FTAI protocols (PRO) were: 1) 7-Day ES + DIV (PRO1, n = 191), 2 mg of EB at DIV insertion on the day (d) -9 and 1 mg of ECP at DIV removal on d -2, and FTAI on d0; 2) 7-Day GnRH + DIV (PRO2, n = 192), 10 µg of GnRH at DIV insertion on d-10, DIV removal on d-3 and GnRH and FTAI on d0; and 3) 7 & 7 GnRH + DIV (PRO3, n = 191), PGF2α at DIV insertion on d-17, GnRH on d-10, DIV removal on d-3 and GnRH and FTAI on d0. Biogenesis-Bagó, Argentina, provided all pharmaceuticals used in this study. All heifers were tail painted at DIV removal to identify those in estrus at FTAI (YES= ≥50% paint removed). Heifers were inseminated by one technician using frozen-thawed semen from a sire at 48-50 or 52-56 h after DIV removal for the estradiol-based or GnRH-based protocols, respectively. Pregnancy was diagnosed by ultrasonography 35 d after FTAI. The effect of FTAI protocols on P/AI was assessed by fitting a logistic regression model that included estrus expression as a main effect and the interaction term between estrus expression and FTAI protocols (PROC GLIMMIX, SAS, 9.4).

Results

Pregnancy per AI did not differ among FTAI protocols (46.1, 53.5 and 53.1% for PRO1, PRO2 and PRO3, respectively; P=0.25). Heifers that expressed estrus had higher P/AI compared to heifers that did not express estrus (60.5 [n=342] vs. 42.2% [n=232]; P<0.0001). In heifers that did not express estrus, PAI did not differ among FTAI protocols; however, in those expressing estrus, PAI was higher in heifers subjected to the PRO2 compared to those subjected to the PRO1 (67.2% [n=192] vs. 54.5 [n=191], P=0.05).

Conclusions

The overall P/AI obtained with both GnRH-based protocols (PRO2 and PRO3) was comparable to that achived with the ES-based protocol (PRO1). A pre-synchronization protocol did not improve P/AI, but the 7-Day GnRH+1X DIV (PRO2) resulted in improved P/AI by 13% compared to the standard 7-Day ES+1XDIV (PRO1) only in heifers expressing estrus. MNC has a fellowship from INTA-CONICET. This study was funded by PICT-2021-I-A-00312 and by IPCVA-2023-29389 to LBF and RLS.

1278 - Precision in Repeat Breeding management in cattle with the assistance of a Behavior Automated Monitoring System

Author: Umesh B. Kumbhar, Juan Pedro Campillo Beneitez, Jantijn Swinkels

Objectives

The aim of the present study is to augment the fertility in repeat breeder cows with hormonal intervention and precision in artificial insemination timing with the help of an automated behavior monitoring system (ABMS).

Material and methods

The study was conducted at Hargun Dairy Farm, Amritsar, Punjab, India with 56 Holstein Friesian crossbred cows from November 2022 to June 2023. The farm has been facing the challenge of repeat breeding syndrome in 13 (23%) animals since 2021. These animals were inseminated three or more times prior to November 2022 on the basis of visual-observed estrus behavior. The average Days in Milk were 605, the average lactation was 2, and the average artificial insemination rate was 4.5 per animal in repeat breeders. All the animals were maintained with the same management and plane of nutrition. The average period of estrus signs was 3.61 days leading to difficulty in the decision of artificial timing by Animal Health Practitioners. To improve the reproductive parameters, the farm installed an automated behavior monitoring system (SenseHub™ Dairy, MSD Animal Health) in September 2022. On the basis of system data, estrus behavior, and prior history, the veterinarian diagnosed 13 animals as repeat breeders with ovulatory defects and selected them for treatment.From the first days of treatment, repeat breeder animals were supplemented with the chelated mineral mixture (VMall ™ Chelated, MSD Animal Health) @ 70g daily, orally, for 30 days. Inj. Sodium salt of 4-dimethylamine,2-methylphenyl-phosphonic acid 0.2g (Tonophosphan®Vet, MSD Animal Health) @ 10mg/kg body weight, intramuscular was administered on an alternate day for 10 days. Artificial insemination was performed at specific times, which were determined with the assistance of the insights provided by the ABMS (SenseHub™ Dairy) which displayed a breeding window (26h) and Heat Index (average 90.93). The Artificial insemination was performed during the later green color part of the breeding window precisely between 10 to 12 hours of the remaining breeding window. At the time of artificial insemination Inj. Buserelin acetate (Receptal®, MSD Animal Health) 2.5 ml was administered intramuscularly. Pregnancy diagnosis was done around 45 days post artificial insemination by per rectal examination method.

Results

Before nutritional treatment, cows manifested mild estrus behaviors with low Heat index scores and watery estrus vaginal discharge based on visual observation. However, after treatment, estrus signs were prominently observed with an average Heat Index of 90.93 percent according to the ABMS. Conception was successfully achieved in all 13 repeat breeder animals following hormonal intervention and precision in artificial insemination timing with the help of an ABMS.

Conclusions

The automated behavior monitoring system (SenseHub™ Dairy) helped the farm to improve the reproductive performance by providing data to assist with the timing of insemination, particularly in repeat breeder animals. The more precise detection of

estrus and timing of insemination were the two crucial parameters to improve reproductive performance. The information provided by the ABMS greatly improved reproductive performance at this farm facility and is a highly recommend tool to improve performance in the management of breeding cattle.

1289 - FIRST CALVES BORN IN MEXICO FROM EMBRYOS OBTAINED FROM 3-MONTH-OLD CALVES USING SEXEDULTRA-4M BEARING CHROMOSOME X SEMEN

Author: HORACIO ÁLVAREZ-GALLARDO, ADRIANA VELÁZQUEZ-ROQUE, FERNANDO VILLASEÑOR-GONZÁLEZ, ELISA OCHOA-ESTRADA, MICHAEL KJELLAND, SALVADOR ROMO-GARCÍA

Objectives

The objective of this research was to evaluate the pregnancy rates and calf sex ratio using fresh *in vitro* produced embryos derived from prepubertal calf oocytes (PCO) fertilized with SexedULTRA-4M bearing chromosome X semen in México.

Material and methods

In vitro embryo production (IVP)

The IVP was performed with a continuous *in vitro* culture system using Vitrogen™ media.

In vitro maturation (IVM)

Under field conditions, PCO were collected by laparoscopic ovum pick up (LOPU) from 3 Holstein calves (3 months) synchronized with an intravaginal device (IVD) CIDR-G (0.3 g) on day 0, on day 2 FSH (120 mg total) was administered IM every 8 h in five applications, 8 h after the fifth application, 400 IU of eCG were applied, and on day 5 the CIDRs-G were removed and LOPU was performed. For the IVM, the cumulus-oocyte complexes (COCs) were selected (only grades 1 and 2) and matured for 24 h at 38.5°C in 5% CO_2 in air and 100% humidity.

In vitro fertilization (IVF) and in vitro culture (IVC).

The IVF process was developed with SexedULTRA-4M frozen semen from a proven bull for IVP, at a concentration of 1 x 10^6 sperm/mL, for 18 h in 38.5 °C, 5% CO_2, 95% air and 100% humidity. Presumptive zygotes were denuded by pipetting and set in IVC until day 7 at 38.5°C, 5% CO_2, 5% O_2 and 90% N_2 at 100% humidity.

Embryo transfer (ET).

For the ET, 20 heifers with a body score condition of 2.5 (on a scale from 1-5) were used as recipients and synchronized with a IVD Crestar (1.3 g) plus 250 µg of gonadorelin on day 0, on day 7 Crestar was removed and a 500 µg dose of cloprostenol sodium was administered, on day 9 a dose of 250 µg of gonadorelin was applied and on day 16 ET was conducted. Only recipients that showed estrus and with a corpus luteum with high irrigation were used.

Pregnancy diagnosis.

Pregnancy rates were evaluated by colour doppler ultrasonography on day 22 and confirmed by ultrasonography on day 40.

Results

There were 18, 25, and 28 viable oocytes collected from donor 1, 2, and 3 respectively. The cleavage rates were 67% for donor 1, 72% for donor 2, and 71% for donor 3. On day 7 of IVC, the blastocysts production was 22.2%, 32%, and 28.5% for donors 1, 2, and 3. For IVP, 4 blastocysts were produced from donor 1 and 8 from donor 2 and 3. After ET,

10 recipients were pregnant at 40 days and 8 calves were born, all the calves were female and 1 died 3 days later. The 1, 4, and 2 calves were weaned from donors 1, 2, and 3, respectively

TABLE 1. SUMMARY OF IVP, BIRTHS, WEANINGS OBTAINED FROM PREPUBERTAL CALVES

DONOR	OOCYTES	CLEAVAGE		BLASTOCYSTS		ET	PREGNANCIES 40 DAYS	BIRTHS	WEANINGS
					n	n	%	n	%
1	18	12	67	4	22.2	4	2	2	1
2	25	18	72	8	32	8	5	4	4
3	28	20	71	8	28.5	8	3	2	2

Conclusions

In conclusion, under the conditions of this research, calves of the desired sex were obtained with acceptable weaning rates. Therefore, we can conclude that it is possible to efficiently produce calves of the desired sex from prepubertal calves in the Holstein breed. Notably, more research is necessary to develop a more practical ovarian stimulation protocol, e.g., one with less injections, and evaluate LOPU-IVP with beef breeds.

1290 - Comparison of the survival of in vitro produced bovine embryos with conventional and SexedULTRA-4M with chromosome X semen to slow-freezing and vitrification

Author: HORACIO ÁLVAREZ-GALLARDO, MICHAEL KJELLAND, MARIO PÉREZ-MARTÍNEZ, ADRIANA VELÁZQUEZ-ROQUE, SALVADOR ROMO-GARCÍA

Objectives

The objective of this research was to evaluate the cryotolerance to slow freezing and vitrification of IVP embryos produced with conventional and SexedULTRA-4M bearing chromosome X semen.

Material and methods

In vitro embryo production (IVP)

The IVP was performed with a continuous *in vitro* culture system using Vitrogen™ media.

In vitro maturation (IVM)

Ovaries were collected from a slaughterhouse and transported (37°C) to the laboratory within 2 h in saline solution (0.9% NaCl) supplemented with penicillin G (100 IU/mL) and streptomycin sulfate (100 µg/mL). For the IVM, the cumulus-oocyte complexes (COCs) were selected (only grades 1 and 2) and matured for 24 h at 38.5°C in 5% CO_2 in air and 100% humidity.

In vitro fertilization (IVF) and culture (IVC)

The IVF process was developed with conventional (CONV) and SexedULTRA-4m (ULTRA-4M) frozen semen from the same bull, both at a concentration of 1 x 10^6 sperm/mL, for 18 h in 38.5 °C, 5% CO2, 95% air and 100% humidity. Presumptive zygotes were denuded by pipetting and set in IVC until day 7 at 38.5°C, 5% CO_2, 5% O_2 and 90% N_2 at 100% humidity.

Cryopreservation

Expanded blastocyst stage embryos (n= 380) quality 1 and 2 (according to the fourth edition of the International Embryo Transfer Society Manual), were divided in 3 groups for each semen type: Control (Cx fresh embryos: n=50), cryopreserved by vitrification (VIT n=70), and slow freezing (SL n=70). The Cx was evaluated only for hatching rates to confirm the proper functioning of the IVP system.

Slow freezing

Blastocysts were subjected to a controlled-rate freezing curve (CL-8800 Cryologic™, Australia) after equilibration for 8 to 10 min in freezing medium with EG (Ethylene Glycol Freeze Plus Vigro™), starting at -6°C (seeding), decreasing 0.5°C/min, ending at -32°C, and then plunging directly into liquid nitrogen.

Vitrification

Blastocysts were vitrified in a protocol with EG and DMSO. Embryos were immersed in vitrification solution 1 (PBS, 10% BSA + 7.5% EG + 7.5% DMSO) for 5 min and then into vitrification solution 2 (PBS, 10% BSA + 15% EG + 15% DMSO + sucrose 0.5 M) for 40 s. Embryos were loaded into Cryotop device (Kitazato™) and stored in LN2.

Thawing/warming.

Frozen embryos were thawed (10 sec on air and 20 sec in water to 30°C); vitrified embryos were warmed in sequentially decreasing sucrose solutions (1, 0.5, 0.25, and 0 M) pre-warmed at 38°C. For both cases, embryos were cultured for 48 h at 38.5°C, 5% CO2, 5% O2 and 90% N2 at 100% humidity.

Statistical analysis

The response variables were re-expansion and hatching rates at 24 and 48 h for each semen type. Statistical analysis was carried out using the Chi-squared procedure on the Jamovi software (version 1.2; The Jamovi project, Sidney, Australia).

Results

Re-expansion rates for SL at 24h were 65.7%, 62.8%; at 48h were 20%, 21.4% respectively for CONV and ULTRA-4M; for VIT 64.2% and 65.7% at 24h and 20% and 18.5% at 48h respectively for CONV and ULTRA-4M. The percentages of hatching for SL at 24 were 42.8% and 44.2%; at 48h were 21.4% and 22.8% respectively for CONV and ULTRA-4M; for VIT 30% and 27.1% at 24h and 11.4% and 14.2% at 48h respectively for CONV and ULTRA-4M. Total hatched rates for SL were 90%, 64.2% and 67.5% for Cx, CONV and ULTRA-4M respectively; for VIT 92%, 41.4% and 41.4% respectively for Cx, CONV and ULTRA-4M. There were no significant differences between the groups and semen type ($P>0.05$) for re-expansion at 24h and 48h. Regarding to hatching rates, there were no significant differences ($P>0.05$) between semen type for both cryopreservation methods at 24h an 48h, however, there were significant differences between cryopreservation method independently of semen type ($P<0.001$).

Conclusions

In conclusion, the ULTRA-4M and CONV had similar cryotolerance concerning SL and VIT. Embryos subjected to SL had better post-thaw viability compared to embryos subjected to VIT, independently of semen type.

1292 - Relation of Dietary Protein Levels on Uterine Proteins During the Reproductive Cycle and Fertility in Dairy Cows

Author: Akbar Pirestani , Reaz Hamedani, Elmira Ziya Motalebipour

Objectives

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Providing human food needs and creating food security is one of the main and most important goals of animal husbandry in developed and developing countries. The biological value of protein materials for dairy cows is directly related to the cow's energy status and the balance of the amino acids they need. There have been various theories on the effect of excess raw protein on the reduction of reproductive power in the reproductive cycle. Furthermore, valuable studies have also been conducted in the field of the effect of dietary proteins with fertility. Current research aimed to explore the effect dietary protein levels on uterine fluid protein across various reproductive cycle stages as well as artificial insemination (AI), fertility rate and pregnancy in dairy cows.

Material and methods

Forty 45-day-old Holstein cows were randomly divided into two groups: one on a 16% protein diet and the other on a 19% protein diet. Following a 14-day adaptation, estrus synchronization was done using heat sensing. Food ration, tailored to factors like weight and age, was administered three times a day as TMR. Uterine fluid samples were collected at each reproductive cycle stage and analyzed for total protein, albumin, alpha (1 and 2), and beta (1 and 2) and gamma globulins.

Results

Statistical analysis with SAS software revealed significant differences, indicating that the 19% protein diet had a more pronounced impact on cervical mucus proteins compared to the 16% protein diet. Examining the results of the comparison of the average dietary protein in the number of AI, fertility rate and pregnancy in this experiment showed no significant difference ($P>0.05$). The results of this experiment showed that the number of AI per pregnancy, the rate of fertility and pregnancy in the 16% dietary protein group was lower than the 19% group, but they did not show a statistically significant difference.

Conclusions

Noteworthy findings included variations in total protein and albumin in the 19% group across cycle stages, emphasizing the importance of protein levels in diet affecting uterine proteins in dairy cows. The non-significance of the data i the number of AI, fertility rate and pregnancy can be caused by factors that have not been investigated in this research, such as the season, weight and possible and mild chronic infections in the

body, stress, the amount of water received the amount of parasitic contamination, the environment.

Keyword: Dietary protein, uterine proteins, fertility, cow's reproductive cycle

1300 - Fertility in dairy cows with different interval between consecutive inseminations

Author: Jorge Antonio Ruiz Perez, Ulises Esau Medina, Noé Juárez, Joel Hernández-Cerón

Objectives
In this study, the hazard of pregnancy was compared in dairy cows with different interval between consecutive inseminations (inter-service intervals).
Material and methods
The study was conducted in a dairy farm located in Querétaro, México. Productive and reproductive records of a total of 2075 lactating cows were analyzed. Cows were milked thrice daily at approximately 8 h intervals; the herd mean projected 305-d lactation yield was used to categorize cows with low (≤11600) or high milk lactation yield (>11600). The voluntary waiting period was 60 days. All the cows received an injection of PGF2α every 14 days starting between 35-40 days postpartum. Estrus was detected by direct observation in the morning and the evening. Cows identified in estrus after day 60 post-partum were inseminated. The pregnancy diagnosis was performed through transrectal palpation between 40-45 days post-service. The intervals between the first and second service were obtained for 1086 cows. Inter-service intervals (ISI) were classified as normal (19-26 days), short (3-18 days), long (27-35 days), double (36-48 days), and greater than 48 days (49-90 days). The final logistic regression model retained service intervals, parity, lactation yield and postpartum health as predictor variables. The parity (primiparous or multiparous), postpartum health (healthy or affected), milk lactation yield (low yield or high yield) variables were considered as binary variables. Additionally, an ordinal regression test was used to compare the distribution of interservice interval proportions among cows of different parity, lactation yield, and postpartum health.
Results
The Odds for pregnancy were reduced in cows that had double ISI (OR: 0.70; 0.51-0.95; $P = 0.026$) and cows that had long ISI (OR: 0.48; 0.28-0.79; $P < 0.05$) in comparation with cows that had normal ISI. The likelihood of pregnancy was similar between cows with short, greater 48 d and normal ISI ($P>0.05$). The pregnancy was most likely in cows with low lactation yield that those with high lactation yield (OR = 1.44; 1.13-1.84; $P < 0.05$). The distribution of ISI´s were 38%, 3%, 7%, 24% and 28% for normal, short, long, double and greater 48 d, respectively. In addition, ISI´s distribution was not affected by parity, lactation yield or postpartum health events ($P > 0.05$).
Conclusions
The findings suggest that cows with long and double inter-service intervals had a lower hazard of pregnancy than cows with normal intervals. On the other hand, the frequency of ISI´s was not affected by parity, milk lactation yield or postpartum health.

1303 - Anogenital Distance in Estradiol/Progesterone (E2/P4)Timed Artificial Insemination (TAI) Protocol in Simmental Cattle

Author: Alberto Gonzalez, Cristian Lopez C, Luis Fernando Escobar, Jaime Eduardo Miranda Muñoz, Bernardo Andres Guerrero Mateus

Objectives

To implement an E2/P4 Timed Artificial Insemination (TAI) protocol in a herd of Simmental and Senepol crossbreeds under a permanent synchronization and resynchronization scheme using color Doppler ultrasound. Evaluate the performance of the hormonal regimen under field conditions in the Colombian coffee-growing region. To describe the anogenital distance to the dorsal commissure and to the clitoris, along with their ratio, in animals subjected to an TAI(E2/P4) protocol).

Material and methods

Ninety-eight adult Simmental females and crossbred Senepol heifers (27 synchronized and 71 resynchronized) were selected under rotational grazing conditions with calves, recruited from 30 days postpartum for synchronization in 10-day E2/P4 protocols. On day 0, 0.6g P4 (Pluselar,Calier) devices accompanied by 2 mg of estradiol benzoate (EB 0,1% Calier) were inserted. Anogenital distance measurements to the dorsal commissure and clitoris were taken with a digital caliper, and body condition was assessed on a scale of 1 to 5. On day 8, the P4 device was removed, and 2 ml of D cloprostenol (Calier), 400 IU of eCG (Vetegon, Calier), and 1 mg of estradiol cypionate (ECP 0,1% Calier) IM were administered. At the same time, crayon marking on the dorsal part of the hip estimated estrus onset. After 48 hours of withdrawal, insemination was carried out using semen from a single bull, with the participation of two experienced inseminators. Doppler ultrasound was performed on day 22 post-TAI using a Mindray DP-50Vet device.The collected data included age in years, number of deliveries, date of the last and penultimate delivery, anogenital distance to the dorsal commissure, anogenital distance to the clitoris, body condition on day 0 and day 22, percentage of shedding, and previous synchronization.

Results

The total percentage of luteal body (CL) positivity in Doppler ultrasound was 41.83% (41/98), 95% CI (32.56-51.73) (Wilson Score). In animals synchronized for the first time, it was 59.25% (16/27), 95% CI (40.73-75.48) and for resynchronized cattle, it was 35.21% (25/71), 95% CI (25.12-46.82) . This difference is statistically significant (p=0.031) Pearson's chi-square. The results indicate a significant association (p=0.026) between being multiparous and the likelihood of being positive on Doppler. The minimum anogenital distance to the dorsal commissure was 33.10 mm, and the maximum was 102.11 mm with a mean of 69.55 mm. For the anogenital distance to the clitoris, the minimum value was 93.85 mm, the maximum was 175.78 mm, and the mean was 139.63 mm. The proportion of distance to the dorsal commissure vs. distance to the clitoris had a minimum of 35.27%, a maximum of 67.25%, and a mean of 49.73%. When categorizing animals by multiparity and resynchronization status, non-multiparous animals that were positive for CL by Doppler ultrasound had a significantly smaller anogenital distance to the dorsal commissure than those reported as negative for Doppler (56.98 mm vs. 65.91 mm) (p=0.046), and similarly, the proportion of the dorsal commissure to the distance to the clitoris (44.64% vs. 49.93%) (p=0.044).

Conclusions

The hormonal package (Calier) is not inferior to results achieved with other hormonal packages in the Colombian market. Multiparous individuals showed a significantly higher CL positivity rate on Doppler than primiparous and nulliparous individuals subjected to the E2/P4 protocol. A significantly smaller anogenital distance to the dorsal commissure was found in Doppler-positive nulliparous and primiparous resynchronized animals compared to Doppler-negative animals subjected to an E2/P4 synchronization protocol.

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1308 - Antibodies against leptospirosis in dairy cows and reproductive parameters

Author: Alejandro Córdova Izquierdo, Alejandro Córdova Izquierdo, Abel E. Villa-Mancera, Armando Gómez-Vázquez, Carlos Bedolla-Cedeño, Jaime Olivares-Pérez, Pedro Sánchez-Aparicio, Ma.De Lourdes Juárez-Mosqueda, Raúl Sánchez-Sánchez

Objectives

Objective: To terminate the presence of antibodies against Leptospirosis in dairy cows and their effect in interval between deliveries (IP), services by conception (S / C) and open days (DA).

Material and methods

Blood samples were taken from the coccygeal vein through the technique of Ojeda et al., (2016), 30 cows of the Holstein breed from a production unit located in the State of Hidalgo, Mexico with a history of abortions, mummifications and embryo reabsorption. The samples were analyzed in the Leptospira laboratory of the Autonomous Metropolitan University-Xochimilco, using the microscopic agglutination technique, which consists of mixing the serum to be studied with leptospires of different serotypes, and then evaluating the degree of agglutination in the microscope (Méndez et al., 2013). Sera were considered positive that at 1: 100 dilution or higher, showed 50% agglutination or disappearance of cells from the field to observation with the dark field microscope. The bacterium that was used was 9 serovars of L. interrogans (Icterohaemorrhagiae, Pyrogenes, Grippotyphosa, Canicola, Pomona, Hardjio, Wolffi, Tarassovi, Bratislava) (Moles et al., 2002). Its relationship with the following reproductive parameters was determined: IP, S / C and DA. The results were analyzed by non-parametric statistics.

Results

In the analysis of the 30 serological samples, 73% (22/30) of positive bovines were found, at one or more serovars of Leptospira interrogans. In the positive sera, three Leptospira interrogans serovars were found: Hardjio, Tarassovi and Hardjio H89, 14% of the animals showed positivity at the three serovars, 32% at two serovars and 55% at one serotype. However, the results suggest an active infection by Tarassovi serovariety since it was found in all cases.

Conclusions

The presence of antibodies against leptospirosis indicates that the condition is present in the production unit of this study, which explains the problems in the reproductive parameters studied IP, S / C and DA.

1309 - Effect of diluent type on sperm quality of refrigerated sheep semen

Author: Alejandro Córdova Izquierdo, Abel E. Villa-Mancera, Armandi Gómez-Vázquez, Carlos Bedolla-Cedeño, Jaime Olivares-Pérez, Pedro Sánchez-Aparicio, Ma. de Lourdes Juárez-Mosqueda, Raúl Sánchez-Sánchez

Objectives
To assess the effect of the type of diluent on sperm quality (motility, viability and acrosomal integrity) of the chilled semen.
Material and methods
Four sheep of the 2 year old English Suffolk breed were used; of which 24 ejaculates were collected through artificial vagina, obtaining 3 to 4 ejaculates per week. Progressive motility, viability, acrosomal integrity (NAR) and pH were assessed. 3 diluents in proportion of 1: 3 were added. The diluents used were: diluent 1 (Rangel, 1985); diluent 2 (egg yolk-Tris-fructose for ram semen -Salamon, 1990) and diluent 3 triladyl base; The semen was kept refrigerated at 4 ° C for 24 hours and the variables mentioned above were evaluated at 0, 2, 4 and 24 hours. The results were analyzed using descriptive statistics, showing averages for each variable.
Results
It was found that the pH did not vary during the 24 hours in the three diluents. Motility and viability were better in the triladyl base diluent for the duration of the experiment. However, acrosomal integrity was better in the first two diluents.
Conclusions
Triladyl-based diluent may present an alternative for the conservation of sheep semen in refrigeration.

1310 - Prostaglandins administration and percentage of pregnancy in heifers in humid tropic

Author: Alejandro Córdova Izquierdo, Abel E. Villa-Mnacera, Armando Gómez-Vázquez, Carlos Bedolla-Cedeño, Jaime Olivares-Pérez, Pedro Sánchez-Aparicio, Ma. de Lourdes Juárez-Mosqueda, Raúl Sánchez-Sánchez

Objectives
To assess the effect of the administration route, intramuscular (IM) and intravulvar (IV) of prostaglandins on the percentage of heifer's pregnancy.
Material and methods
The study was carried out in a humid tropic cattle production unit in the State of Tabasco, Mexico. Selection of animals: A total of 29 simbrah heifers were selected, under conditions of extensive management, the selection criterion was heifers with the presence of corpus luteum (CL) at rectal palpation with a body condition of 2.5 and 3 (scale 1-5), with an average age of 1.5 years and with feeding in conventional grazing. For the study, the animals were divided into two groups by applying synthetic prostaglandins in two ways, IM and IV. Treatment 1 (T1): 13 heifers were used which were given on day 0 an intramuscular injection of PGF2∝ (Chlorprostenol 530 mcg / 2 ml) which is synthetic and analogous, structurally related to PGF2∝. Day 0 is counted as the day of application of PGF2∝ to heifers that presented functional CL to rectal palpation, 96 hours were expected to carry out artificial insemination (AI) at a fixed time, and the diagnosis of pregnancy was made by rectal palpation at 45 days after AI. Treatment 2 (T2): 16 heifers were used which were given on day 0 an intravulvar injection of PGF2∝ (Dinoprost 10mg / 2ml) and the procedure used in T1 was performed.
Results
The results were: 77% and 93% of gestations for group T1 and T2, respectively. In conclusion, the best gestation results were obtained when the IV administration route was used.
Conclusions
The used type of prostaglandin (dinoprost) by IV route improved the gestation percentage compared to that obtained with cloprostenol by IM route, which supports the works that broadly affirm that the use of estrous synchronization schemes artificial insemination in fixed time, is a practical way to be used in animal production units of cattle under humid tropic conditions and with extensive management system in conventional grazing.

1314 - There is a genetic cause of intestinal atresia in cattle – new study reveals sire link

Author: John Mee F, Orla Keane, Tara McCarthy, James Hanrahan, Daragh Matthews, John McEwan, Suzanne Rowe, Jonathon Kenneally

Objectives
Intestinal atresia is one of the most common congenital defects recorded in calves (Mee et al., 2023). However, the etiology of this common defect has eluded scientists with competing hypothesis, both genetic and non-genetic, proposed. Hence, the objectives of this study were to establish both the incidence and risk factors for intestinal atresia in Irish dairy herds and to detect associated genomic variants.
Material and methods
Cases (n=197) of atresia (jejuni, coli or ani) from 40 dairy farms were examined by necropsy where muscle was collected for DNA extraction. Data on all calves born (n=56,454) and their dams/sires during the 8-year study were retrieved. A dataset of high-density genotypes (196 cases, 1,071 controls) was used for a genome wide association study (GWAS). The phased genotypes of two paternal half-sib Jersey sires with a high proportion of affected progeny were used to identify shared paternal haplotypes.
Results
In total 197/56,454 calves had atresia; 0.35% (0-1.3% by farm). The majority of cases (83%) had jejunal atresia. Atresia was twice as common in male as in female calves ($P < 0.0001$), and was more common in progeny of older than in progeny of first or second lactation cows ($P < 0.001$). Year and farm were also risk factors ($P < 0.05$). The incidence of atresia was highest among the progeny of related Jersey sires, suggesting a gene for atresia was segregating within this family. There was no evidence of a sire-effect among the progeny of Holstein-Friesian sires. However, a case-control GWAS identified 31 SNP in 18 loci as associated with the defect in this breed.
Conclusions
The jejunum is most common site of atresia in Irish dairy calves. Sex, parity, farm and year are risk factors. This study has shown that atresia in calves is not a simple Mendelian trait as previously reported, but a complex multigenic disorder.

Reference
Mee J. F., Murphy, D. and Curran, M. (2023) Bovine congenital defects recorded by veterinary practitioners. *Reproduction in Domestic Animals*, 00:1–9, DOI: 10.1111/rda.14501

1329 - Veterinary practitioner-recorded bovine congenital defects using a new app - Final

Author: John Mee F, Donal Murphy, Mike Curran

Objectives

Congenital defects may be defined as abnormalities of structure and function present at birth (1). The objective of the project was to field-test a mobile phone app designed for use by veterinary practitioners to collect clinical case data on congenital defects during routine farm visits

Material and methods

A 15-question questionnaire was designed, piloted with veterinary practitioners, and then formatted as a mobile phone app using Typeform (www.typeform.com). In addition, up to three photographic images or videos could be recorded per case. The app was provided to all veterinary practitioners (n=190) in an Irish practice group in December, 2020. Epidemiological information and images were collected during routine farm visits (mainly calvings). The questionnaire data and the images were stored in a secure azure cloud server and presented as an interrogatable dashboard using Microsoft Power Bi. In total, 191 congenital defect cases were categorised by the body system/s affected (2).

Results

In total, 59 vets in 28 XLVets Ireland veterinary practices distributed nationally participated in the project across three years (2020-2022). The number of cases/vet and /practice varied between 1 and 23 and 1 and 43, respectively.

The three most commonly recorded individual defects were intestinal atresia (24.1%), *Schistosomus reflexus* (20.4%) and ankylosis (6.8%); multiple defects were recorded in 13.1% of cases. Intestinal atresia included atresia of the intestines (43) and atresia ani (3). Multiple defects most commonly affected the musculoskeletal system (22/25 cases) and of these, those involving ankyloses (11) and palatoschisis (6) were the most common.

The majority of dams were pluriparae (141), of a dairy breed (138); the most common dam breed was Holstein-Friesian/cross (132). The majority of sires were of a beef breed (93), with the remainder dairy (81) and other. The most common sire breeds was Holstein-Friesian/cross (81) and the majority of cows were bred by artificial insemination (77). There was a similar number of male (43) and female cases (42) but the majority of cases had an unrecorded sex (106). The majority of cases were singletons (178) with the remainder twins (13). Gestation was full term in the majority of cases (180). The majority of cases were assisted at calving [Caesarean section (46), non-surgical assistance (41) or foetotomy (20)]. The majority of cases were recorded at birth (76) with the remainder in the first day (21).

Conclusions

This study highlighted two congenital defects of cattle which are often considered as sporadic in occurrence and of low prevalence. Based on the findings reported here, both intestinal atresia and *Schistosomus reflexus* need to be viewed in a new light as clinically important bovine congenital defects warranting preventive strategies at farm and national levels. The results of this study also indicate that veterinary practitioner

surveillance using mobile phone apps could be expanded to include other clinically important conditions and in other countries.

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1330 - Estimation of gestational age in bovine aborted and stillborn fetuses by fetal morphometry - Final

Author: Paulina Jawor, John Mee F

Objectives

In the absence of accurate AI or natural service dating it can be difficult to estimate fetal gestational age after the first trimester of pregnancy. Hence, the objective of this study was to use foetal morphometrics to predict gestation length (GL) in aborted and stillborn fetuses from dairy cows.

Material and methods

Data (GL, calf breed and necropsy records) from 1,295 single birth cases of bovine abortion and perinatal mortality on Irish dairy farms were examined. Gestation length varied between 128 and 316 days. The following body morphometrics were recorded at necropsy: DD - digital diameter of fetlock, Girth - behind the shoulder, CRL (crown rump length) pollc -curved from base of tail to crown, CRL eye - curved from base of tail to eye, Str CRL -straight from rump to crown and body weight. Calf breed was categorised as Je = Je or Jex sire or dam (n=394); Non-Je = other dairy breeds or crosses (n=450); Beef = beef sire on non-Je (n=142); un = unclear (n=10). Only records from Je and Non-Je calves were used. Data analysis was performed using Python 3.9.7 and linear regression with Statsmodels package. The final dataset (844 records) was divided into train (80%) and test/validation (20%) sets.

Results

Morphometric variables were highly autocorrelated (0.86-0.98), hence models with one morphometric variable and breed category were built. While breed category was statistically significant, it had little impact on the output values. Girth, DD and sCRL had the highest R^2 in train and test datasets (0-48-0.63) combined with lowest mean absolute error (MAE), (mean difference in predicted days from GL) (10.3) and lowest percentage of mismatch cases (more than +/-24 days from GL), (9.5-10). Body weight had the poorest associations with GL.

Conclusions

Bovine gestation length, from the fourth month of pregnancy onwards, could be predicted with different morphometrics within approximately $\pm$10 days in dairy calves.

1347 - Evaluation of clinicians and farm veterinarians' clinical approaches to the calving process in Turkey: A questionnaire study

Author: Pelin Erden, Mine Aydemir, İrem ÇAKMAK, Gülnaz MECİTOĞLU, Ahmet GÜMEN, Abdulkadir KESKİN, John F. Mee

Objectives

This study was designed to evaluate the interventions and approaches of clinicians (clinic/veterinary hospital owners) and farm veterinarians (full-time employees on the farm) to management before, during, and after calving.

Material and methods

A questionnaire was sent to a total of 490 veterinarians working in different provinces of Turkey; 430 veterinarians working in 69 different provinces responded (88% response rate) and were included in the study (n=339 clinicians and n=91 farm veterinarians). The data were collected by online questionnaire (Google Forms®). There were 30 questions; to determine the demographic profile (n=10); and veterinarians' clinical approaches to the calving period (n= 20). The Chi-Square test and Mann-Whitney U test were used to analyse the correlations between variables and to compare responses from clinicians and farm veterinarians.

Results

In total, 96.3 % of the veterinarians participating in the survey were male and 3.7 % were female and the mean (sd) age of the participants was 37±8.7 years. The average duration of professional experience was 12.7±8.7 years. In answer to the question as to whether one should intervene prophylactically at calving, the farm veterinarians (72.5 %) were more likely not to intervene prophylactically (p = 0.001) compared to clinicians (47.5 %). In answer to the question as to whether or not to wait for spontaneous calving where everything seems to be going normally (when the forelimb and nose were visible at the vulvar lips), 69.4 % of clinicians and 96.6 % of farm veterinarians preferred to wait (p < 0.001). When asked about stress due to the calving case difficulties and environmental conditions (barn conditions, animal owner etc.), more clinicians responded that they experienced stress (57.8 %) compared to farm veterinarians (43.3 %), (p = 0.049). Veterinarians were asked about the frequency of calving jack usage; more clinicians used a jack than farm veterinarians (p = 0.008). The approach to suspending the calf to remove birth fluids was evaluated. More farm veterinarians (34.4 %) did not use this procedure (p = 0.001) compared to the clinicians (17.1 %). Overall, 19.7 % of veterinarians administered analgesic/anti-inflammatory drugs to the cow after eutocia; 80.3 % did not. More farm veterinarians (87.9 %) than clinicians (80.2 %) did not administer these pain medications (p<0.04). Overall, 38.3 % of vets administered antibiotics to cows after eutocia, 42.1 % of clinicians and 24.2 % of farm veterinarians (p < 0.002). While 86.2 % of veterinarians administered antibiotics to cows after dystocia, this rate was higher (p < 0.02) in clinicians (88.4 %) than in farm veterinarians (78.0 %). Overall, 48.2 % of veterinarians did not perform any prophylactic practices against retained fetal membranes (RFM) after eutocia, and this did not differ between clinicians and farm vets. In contrast, overall, 75.8 % of veterinarians performed prophylactic measurements to prevent RFM after dystocia and this did not differ between clinicians and farm vets. Regarding the clinical approach to RFM after calving; 39.2 % of veterinarians preferred manual removal, 36.4 % preferred to leave the membranes if

manual removal was unsuccessful, and 24.4 % chose to leave the fetal membranes without manipulation. Nonetheless, 70.0 % of the veterinarians performed manual removal of the fetal membranes before the third day after calving, and almost all of the veterinarians (99.0 %) administered antibiotics in cases of high body temperature (>39.5 °C) after manual removal or in cases where the fetal membrane was left without manipulation.

Conclusions

This study demonstrated significant differences in the approaches of clinicians and farm vets to some aspects of calving cases. Clinicians were more proactive to intervene during calving and were more likely to use a calving jack, analgesia and antibiotics than farm veterinarians. Further studies should seek to explore why these differences exist and what implications they have for calving case management.

1350 - Superovulation of lactating Holstein cows with a single dose of recombinant bovine FSH in a commercial dairy setting in Brazil

Author: Carlos Rodrigues Alberto, Bruna Catussi, Lígia Rebeis, Sofia Albertini, Pedro Pinho, Federico Randi, Pietro Baruselli

Objectives

Until recent times, gonadotropin FSH for superovulation in cattle was primarily extracted from the pituitary of slaughtered animals, mainly pigs. However, this method presented several drawbacks, including potential contamination, pathogen transmission and batch-to-batch inconsistency. Recently, alternative production methods utilizing recombinant technology have been licensed to produce a bovine recombinant FSH homologue. Therefore, the aim of this study was to evaluate the effectiveness of a single injection long-acting recombinant bovine FSH on superovulation for *in vivo* embryo production in lactating Holstein primiparous cows.

Material and methods

Twenty primiparous lactating Holstein cows [days in milk (DMI)= 99.8 ± 6.80 and milk production (MILK) per day = 53.8 ± 1.53 kg], sourced from Agrindus farm (Descalvado, SP, Brazil) at a random stage of their estrous cycle were subjected to a synchronization program. On D0, cows received 2 mg of estradiol benzoate and the introduction of a progesterone-releasing intravaginal device (1,55g of progesterone). Four days later, the cows received 6ml of recombinant bovine FSH (Zimbria®, Ceva Sante Animale, France); equivalent to 300 mcg of rb-FSH. On day 6 a.m., the first injection of PGF 2alpha (150 mcg of D-cloprostenol) was administered, followed by a second injection of PGF 2alpha on day 6 p.m. The progesterone device was removed on day 7 p.m., and on day 8 p.m. all the cows received GnRH (20 mcg of buserelin acetate). Donors received two timed artificial inseminations, one on day 9 a.m. and the second one on day 9 p.m. All the cows were flushed on day 15 p.m. The number of viable embryos, degenerated embryos and unfertilized oocytes were collected and correlated with the number of corpora lutea (CL) present on both ovaries at flushing time, evaluated by transrectal ultrasonography. Cows were classified as high DIM (121.7 ± 8.32 days in milk, n=10) and low DIM (77.9 ± 4.44 days in milk, n=10). Also, cows were classified as high MILK (59.5 ± 1.14 Kg/day, n= 10) and low MILK (48.1 ± 1.18 Kg/day) to evaluate the number of CL, number of viable embryos, recovery rate, unfertilized embryo rate, and degenerate embryo rate. Statistical analyses were performed by SAS Glimmix procedure (v9.4).

Results

No interaction was found between DIM and MILK for any variables (P > 0.05). The number of CL did not differ between DIM groups (High DIM= 12.7 ± 1.9 vs. Low DIM= 13.8 ± 3.3; P= 0.73) nor between MILK groups (High MILK= 12.3 ± 2.7 vs. Low MILK= 14.2 ± 2.7; P= 0.47). No differences were observed for number of viable embryos between High and Low DIM (5.90 ± 1.4 vs. 4.10 ± 1.1; P= 0.35) nor between High and Low MILK (5.70 ± 1.40 vs. 4.30 ± 1.08; P= 0.54). The recovery rate was higher in High than Low DIM (76% vs. 63%; P= 0.03). However, no difference was found for MILK (High MILK= 73% vs. Low MILK= 67%; P= 0.32). The unfertilized embryo rate was similar between High or Low DIM groups (27% vs. 21%; P= 0.97) and between High or Low MILK groups (24% vs. 25%; P= 0.25). Furthermore, the degenerated embryo rate did not differ between High and Low DIM (13% vs. 19%; P=0.37) nor between High and Low MILK (8% vs. 23%; P= 0.16).

Conclusions

The study investigated the efficacy of a single injection of long-acting recombinant bovine FSH for superovulation in lactating Holstein primiparous cows. The study showed the efficacy of the product in inducing multiple ovulations and multi embryonic developments. Results indicated no significant differences in the number of viable embryos, unfertilized embryos, or degenerated embryos between High and Low DIM or MILK groups. However, a higher recovery rate was observed in cows with High DIM. These findings suggest that the recombinant bovine FSH homologue (Zimbria®, Ceva) offers a viable alternative to traditional pituitary extracts which required multiple injections for superovulation. Furthermore, the recombinant molecule can bring benefits in terms of safety and consistency. Further research and larger-scale studies are warranted to validate and expand upon these preliminary findings.

1361 - Fertility performances of lactating dairy cows submitted to timed artificial insemination or timed embryo transfer with fresh or frozen in vitro produced embryos in a seasonal pasture-based system

Author: Alan Crowe, Dr. Federico Randi , Jose Maria Sanchez, Stephen G. Moore, Michael McDondald, Rafaela Rodrigues, Maria Fernanda Morales, Leandro Orsi De Freitas, Maria Belen Rabaglino, Patrick Lonergan, stephen T. Butler

Objectives
The aims of this study was to compare pregnancy per service (P/S) in lactating dairy cows following timed artificial insemination (TAI) or timed embryo transfer (TET) using either fresh or frozen in vitro produced (IVP) embryos.
Material and methods
Oocytes were collected once per week for up to 9 weeks using transvaginal ovum pick-up from elite dairy donors (ET-DAIRY; n = 40; Holstein-Friesian and Jersey) and elite beef donors (ET-ELITE-BEEF; n = 21; Angus). Both ET-DAIRY and ET-ELITE-BEEF donors included both heifers and cows. Another proportion of oocytes were collected from the ovaries of beef heifers of known pedigree following slaughter (ET-COMM-BEEF; n = 119). Following in vitro maturation, fertilisation and culture, single Grade 1 blastocysts were transferred either fresh or following freezing and on-farm thawing into lactating, Holstein-Friesian, dairy cows which had been synchronised with a 10-d PRID-Ovsynch protocol.
On day 0, a 2-mL i.m. injection of GnRH analogue (Ovarelin, 100 µg of gonadorelin diacetate tetrahydrate; Ceva Sante Animale, Libourne, France) was administered, and a progesterone-releasing intravaginal device (PRID Delta; 1.55 g Progesterone, Ceva) was inserted. On day 7, a 5-mL i.m. injection of PGF2α (Enzaprost, 25 mg of dinoprost trometamol; Ceva) was administered. On day 8, a second 5-mL i.m. of PGF2α was administered and the PRID was removed. On day 9.5 (32 h after PRID removal), a second i.m. injection of GnRH was administered. On day 10, 243 cows received AI (16 h after the second GnRH), and 863 cows were assigned to receive ET on day 17. All the animals were blood sampled on day 17 to evaluate serum P4 concentrations.
Pregnancy rate was determined between Day 32-35 after synchronised ovulation using transrectal ultrasonography. Between Day 62-65 after synchronised ovulation, pregnancy was confirmed to determine embryonic loss.
Pregnancy Data were analysed using generalised linear mixed models including service treatment (TAI vs. TET) as a fixed effect.
Results
Mean pregnancy/service at Day 32 was not different between AI (48.8%) and ET (48.9%) and did not differ between dairy and beef embryos (50.3% vs 48.1% respectively).
P/S was less (P = 0.003) following transfer of frozen embryos compared with fresh embryos (41.6% vs 56.1% respectively). Pregnancy loss between day 32 and 62 was greater (P=0.003) for ET (15.1%) compared with AI (4.7%), with greater losses observed for frozen beef (18.5%), fresh beef (17.3%) and frozen dairy (19.2%) compared with fresh dairy (6.0%).
Serum P4 concentration on d 7 was associated with P/S at d 32 and d 62.

Cows in the quartile with the least serum P4 concentrations (Q1) had less probability of being pregnant on d 32 (33.4%) compared with cows in the 3 upper quartiles for serum P4 (45.7%, 55.6% and 61.2 for Q2, Q3 and Q4, respectively).

Conclusions

In conclusion, pregnancy/service event on Day 32-35 was similar for TAI and TET, although 9.6% of cows initially synchronized for ET were rejected. Of cows that were pregnant on day 32, pregnancy loss was greater for TET than for TAI. P4 concentrations at day 7 was positively correlated with pregnancy both at D32 and day 65.

1362 - Use of a strategic injectable trace mineral supplementation to improve Holstein cows' reproductive efficiency and survival in Northern Mexico

Author: Marcello Guadagnini, José Eladio Barraza Rodríguez , Julio Prado

Objectives

A trace mineral optimal status is key for reproductive success in cattle. Zinc, Copper and Manganese deficiencies have been associated with reproductive impairment. An alteration of the trace mineral status may occur in dairy cows despite adequate oral supplementation, often due to the presence of mineral antagonists in forages or drinking water. The "Comarca Lagunera" region in northern Mexico has been documented to have high sulfur and iron levels in water that could negatively affect oral copper absorption. The aim of the present work was to assess if ensuring an optimal trace mineral status with a strategic trace mineral injection after calving could improve reproductive efficiency and survival in Mexican Holstein cows.

Material and methods

Three Holstein confined herds located in the "Comarca Lagunera" region in Northern Mexico have been enrolled in the study. A total of 517 among primiparous and multiparous cows have been randomly assigned after calving to treatment or control group. At the enrollment in the study, animals belonging to the treatment group (MM) (n=258) received one injection (1 ml/90 Kg) of a multimineral solution containing Zinc (60mg/ml), Copper (15mg/ml), Manganese (10mg/ml) and Selenium (5mg/ml) (Multimin 90®, Axiota Animal Health) at 56±1 days in milk (DIM) in Herd 1, 36±1 DIM in Herd 2 and 3. Control group cows (CON) (n=258) remained untreated. Each herd had an even distribution of animals among treatment groups. After a voluntary waiting period (70 days for Herd 1 and 50 days for Herd 2 and 3) animals were inseminated upon estrus detection. Animals were observed till 200 days in milk and reproductive efficiency was calculated using Kaplan-Meier curves for time to conception by treatment group. The association of treatment with pregnancy risk in the first 200 DIM was calculated using a Cox's proportional hazard model where dependent variables were herd, parity number (1, 2, 3+), DIM at enrollment, month/year of calving, treatment, and treatment by herd and treatment by parity interactions. The proportion of pregnant cows at 200 DIM was calculated and compared among treatment groups with a simple logistic regression model. Moreover, Kaplan-Meier curves were used to address cows' survival by treatment group from the time of enrollment till 150 DIM. Culled (sold and died) cows' percentage from the enrollment till 150 DIM was also calculated, excluding cows that left the herd for dairy purposes. A Cox's proportional hazard model for the risk of culling was built including the same dependent variables as the fertility model. The statistical analysis was performed with JMP 16® (SAS). Significant differences were considered for $p \leq 0.05$.

Results

Overall, 198 primiparous cows, 179 second calving cows and 140 cows with 3 or more calvings were included in the study. Treatment groups did not differ for parity distribution (p=0.42), nor for DIM at the enrollment (p=0.55). Kaplan-Meier curves calculated a median time to conception significantly lower for MM compared to CON cows (117 days vs 138 days, respectively; p=0.04). In the Cox's proportional hazard

model, treatment was significantly associated with pregnancy risk (p=0.02), with MM cows having 1.33 (HR 1.04-1.70) times greater odds of becoming pregnant compared to CON cows. Both interactions of treatment by herd and treatment by parity were not significant and were removed from the model. The proportion of pregnant cows at 200 DIM was significantly higher in the MM group (75.3%) compared to the CON group (64.2%) (p=0.01). Kaplan-Meier curves by treatment showed that CON cows exit the herd significantly faster than MM cows (p=0.008). Cox's proportional hazard model for survival till 150 DIM calculated greater odds of being culled for CON animals 2.19 (HR 1.15-4.15) compared to MM animals. Again, the interaction of treatment by herd and treatment by parity were not significant and were, therefore, removed from the model. The proportion of culled cows at 150 DIM in the MM group was lower (5.4%) compared to CON (12.0%) (p=0.008).

Conclusions

This study's results demonstrated that, in certain environmental conditions where antagonism is very likely to negatively impact the trace mineral status, dairy cows' fertility and survival can be improved with a strategic trace mineral injection during the voluntary waiting period.

1397 - Comparative Analysis of Automated Behavior Monitoring Systems (ABMS) versus Visual Observation: Impact on Reproductive Parameters and Farm Profitability in Dairy Heifers

Author: Dogus Ozkan, Juan Pedro Campillo Beneitez, İldeniz Dünder , İhsan Ballan

Objectives

In recent years, dairy farms have increasingly embraced a range of technological solutions to enhance efficiency and improve the quality of life by delivering insights that assist farmers in making decisions in reproduction, health and nutrition. Among these, automated behavior monitoring systems (AMBS) have become a popular choice in dairy farming. This study aims to compare the effects of utilizing an AMBS versus visual observation in heifers on key reproduction parameters, including Conception Rate, Number of Semen Per Pregnancy, and Pregnancy Age.

Material and methods

The study was conducted at Lidersan dairy farm in Gaziantep, Türkiye, between January 2022 and April 2022. The farm has in total 8,000 dairy animals.

For this trial, the breeding heifers were randomly assigned to two groups: the Behavior Monitoring Group (BMG) and the Control Group (CG). The BMG included 91 heifers that were wearing a behavior monitoring neck tag (SenseHub Monitoring Neck Tag, MSD Animal Health) for helping the farmer to detect the heifers in heat. The system heat alerts were displayed in an Automated Behavior Monitoring System (ABMS) (SenseHub Dairy, MSD Animal Health). The CG consisted of 98 heifers without wearing behavior monitoring tags. Both groups of heifers were 13 months old and were housed under identical conditions with the same feeding rations.

In the BMG, insemination was solely based on the ABMS alerts, whereas the CG relied on visual observation. The BMG started insemination at 361 days old, while the CG began at 357 days old.

Results

The study compared two groups of heifers: Behavior Monitoring Group (BMG) and Control Group (CG). The results showed that the BMG had a higher Conception Rate (CR %) of 51.7% compared to the CG with a CR % of 42.86%. The number of artificial Inseminations per pregnancy was slightly higher in the CG heifers (2.1) compared to the BMG heifers (1.94). Additionally, the pregnancy age of the CG heifers was longer, with an average of 420 days compared to 408 days for the BMG heifers. Although the age at first insemination for the CG was 4 days earlier than the BMG, in average the control group heifers became pregnant 12 days later. The CG had in total 16 days later the age for first calving compared to the BMG. This indicates that the CG spent 16 more days being fed as heifers.

The financial implications of these results were also investigated. The exchange rate used in the analysis was 20.89 TL per currency unit. The daily feeding cost for heifers older than 12 months was found to be 53.89 TL ($2.58), while the feeding cost for pregnant heifers for more than 7 months was 61.87 TL ($2.96). The cost of semen was 1357.85 TL ($65.00) per unit.

Based on the difference if the heifer age at conception (12 days) and the average difference number of semen per pregnancy (0.16), the study calculated the feed saving

per animal to be 646.68 TL and the semen saving per animal to be 217.25 TL. This resulted in a total saving per head of 863.93 TL ($41.35).
When extrapolated to the total number of breeding heifers annually (2,114), the study estimated that the total saving per year would be 1,826,34 TL, which is equivalent to approximately 87,426 USD.
These findings indicate that the heifers in the BMG exhibited improved reproductive performance and potential cost savings compared to the heifers in the CG.

Conclusions

In accordance with the study findings, the implementation of automated behavior monitoring systems (AMBS) has demonstrated the potential to yield reproductive performance outcomes that are comparable to those achieved through visual observation. Additionally, the study observed that cows monitored with the reproduction monitoring system experienced a shorter time to pregnancy. Although the primary objective of this study was not to assess the impact on workflow efficiency and labor, it is suggested that farms could potentially benefit from the implementation of AMBS in these aspects. In addition to these reproductive benefits, the study also indicated that the implementation of monitoring systems had a positive impact on the profitability and efficiency of the entire herd.

1401 - The use of silver nanoparticles in mastitis therapy

Author: Jan Twardoń , Monika Szpringiel, Anna Wanecka, Jarosław Król

Objectives

Mastitis is one of the most common diseases of dairy cattle which contributes to abundant antibiotics use [2]. In order to limit the use of such substances, the need to search for alternative forms of mastitis treatment arises. One of such methods could be using preparations with silver nanoparticles. Nanosilver are small particles of silver which manifest strong antibacterial properties [1]. The following study determines the usefulness of silver nanoparticles in mastitis therapy.

Material and methods

In the following study 410 cows from 2 farms were examined (clinical examination of udder and milk). On both objects milking took place twice a day and annual mastitis incidence was 7-8%. The clinical examination of udder comprised observation and palpation. Milk samples were collected from each quarter separately and the examination consisted of:

- macroscopic examination,
- California Mastitis Test,
- microbiological examination - culture - performed on milk samples collected from cows with positive results from the previous tests,
- antibiogram from positive samples to determine bacteria susceptibility for antibiotics.

The microbiological examination was performed on 360 samples and 183 of them turned out to be positive. Milk samples (50 µl) were inoculated on tryptic-soy agar (BioMaxima, Poland), supplemented with 5% defibrinated sheep blood, on MacConkey Agar (BioMaxima) and on Sabouraud Dextrose Agar (Oxoid, UK). Plates were inoculated aerobically for 24 and 48 h at 37°C. Isolated microorganisms were tentatively identified based on colony and cell morphology, Gram reaction and catalase activity. Catalase-positive and catalase-negative Gram-positive cocciwere further examined by means of ID 32 Staph and API 20 STREP identification systems (bioMérieux, France), respectively. All potentially pathogenic bacteria were subject to antimicrobial susceptibility test (using the disk diffusion method) with the following antimicrobial agents: amoxicillin with clavulanic acid, cloxacillin, lincomycin with neomycin, penicillin, and a combination of penicillin, novobiocin, neomycin and streptomycin.

Obtained resulted enabled division of enrolled cows and quarters into the following groups:

- group 1 (n=68) received intramammary product containing 250 ppm of silver nanoparticles twice (every 12 hours),
- group 2 (n=30) received intramammary antibiotic chosen on the basis of antibiogram, according to manufacturer's recommendations
- group 3 (n=20) control group which was not given any medicine.

The dose of nanosilver was assessed in in vitro tests (data not published). After 14 days, milk samples from enrolled animals were collected again in order to assess the efficacy of treatments. In order to do so, the same tests were performed, excluding antibiograms.

Results

The control performed two weeks after intramammary use of nanosilver product, antibiotics or no treatment gave the following results:
- group 1, in which cows received twice preparation with 250 ppm of silver nanoparticles
-> improvement: 55,9%
-> setback: 17,6%
-> no changes: 26,5%
- group 2, in which animals were given antibiotics in the form of intramammary tubes:
-> improvement: 76,7%
-> setback: 0%
-> no changes 23,3%
- group 3, which was a control group:
->improvement: 45%
-> setback 25%
-> no changes 30%.

Conclusions

Silver nanoparticles in concentration 250 ppm, administered twice every 12 hours is a useful method of mastitis treatment. Moreover, it can be considered as an alternative for antibiotic treatment. It is an effective and simple way to treat mammary gland inflammation. Silver nanoparticles have antibacterial properties which can be effective in treatment of bacterial inflammatory processes. Unlike antibiotics, silver nanoparticles have no influence on the withdrawal period of milk and tissue.

1444 - Fertility of postpartum Bos taurus beef cows receiving estradiol/progesterone-based fixed-time artificial insemination protocols with intravaginal devices containing 0.5 or 1.9 g of progesterone

Author: Gilson Antonio Pessoa, Izaias Claro Junior, Jose Luiz Moraes Vasconcelos, Francisco Lopes Junior, Normelio Alves, Ocilon Gomes de Sa Filho, Pedro Levy Piza Fontes

Objectives

The aims of this study were to 1) compare circulating concentrations of progesterone (P4), follicle diameter, estrus expression, and pregnancy rates of *Bos taurus* beef cows submitted to estradiol/P4-based fixed-time artificial insemination (FTAI) protocols with intravaginal devices containing 0.5 g vs. 1.9 g of P4, and 2) evaluate the impact of earlier administration of prostaglandin $F_{2\alpha}$ (PGF) on the fertility of cows receiving devices containing 0.5 g of P4.

Material and methods

Postpartum *Bos taurus* beef cows (n = 1,926; 829 primiparous and 1,097 multiparous; 479.3 ± 67.2 kg body weight; body condition score 3.39 ± 0.63 in a 1 to 5 scale) from seven locations in Rio Grande do Sul State (Brazil) were randomly assigned within location to 1 of 3 treatments: **1)** an intravaginal insert containing 1.9 g of P4 (CIDR®, Zoetis) + 2 mg of estradiol benzoate (2 mL i.m. of Gonadiol®, Zoetis) on day 0; 12.5 mg of dinoprost tromethamine (2.5 mL i.m. of Lutalyse®, Zoetis) on day 7; CIDR® withdrawal + 300 IU of equine chorionic gonadotropin (1.5 mL i.m. of Novormon®, Zoetis) + 0.6 mg of estradiol cypionate (0.3 mL i.m. of E.C.P.®, Zoetis) on day 9; FTAI on day 11 (48 ± 2 h after CIDR® withdrawal; **CIDR-PGF7**, n = 749); **2)** a synchronization of ovulation protocol similar to CIDR-PGF7, however using an intravaginal device containing 0.5 g of P4 (DIB® 0.5, Zoetis; **DIB-PGF7**, n = 594); **3**) a synchronization of ovulation protocol similar to DIB-PGF7, however with Lutalyse® treatment on day 9 instead of day 7 (**DIB-PGF9**, n = 583). On day 9, in a subset of cows (n = 332), blood samples were collected for evaluation of serum concentrations of P4 and transrectal ovarian ultrasonography was performed to determine dominant follicle diameter. Estrus expression was evaluated between days 9 and 11, and pregnancy diagnosis was performed 30 days after FTAI by ultrasonography. Data were analyzed using PROC GLIMMIX (binomial variables) or PROC GLM (continuous variables) of SAS and means compared when treatment effects detected.

Results

Serum concentrations of P4 on day 9 and pregnancy rates were greater ($P < 0.01$) in cows receiving CIDR-PGF7 (2.0 ± 0.09 ng/mL; 54.9%) than in cows receiving DIB-PGF7 (1.2 ± 0.10 ng/mL; 40.1%) and DIB-PGF9 (1.5 ± 0.12 ng/mL; 45.9%) but did not differ between DIB-PGF7 and DIB-PGF9 treatments.

Dominant follicle diameter on day 9 and the percentage of cows expressing estrus within 24 h after P4 devices removal were greater ($P < 0.01$) in cows receiving the DIB-PGF7 (13.0 ± 0.26 mm; 19.3%) than in cows receiving CIDR-PGF7 (12.0 ± 0.26 mm; 9.6%) and DIB-PGF9 (12.1 ± 0.26 mm; 11.7%), but not different between CIDR-PGF7 and DIB-PGF9. The percentage of cows expressing estrus between 24 and 48 h after P4 devices removal and the overall estrus expression during the 48 h period between P4 devices removal and FTAI were greater ($P < 0.01$) in cows receiving CIDR-PGF7 (69.5% and 79.9%, respectively) than in cows receiving DIB-PGF7 (51.9% and 72.6%, respectively) and DIB-

PGF9 (54.6% and 67.4%, respectively), but similar between DIB-PGF7 and DIB-PGF9 treatments.

No effects of parity or interaction between treatment and parity were found in any of the dependent variables.

Conclusions

Utilization of an intravaginal device containing 1.9 g of P4 in *Bos taurus* cows resulted in increased overall estrus expression, percentage of cows expressing estrus in the appropriated time, and pregnancy rate, whereas a device containing 0.5 g of P4 hastened follicular development prior to its removal, resulting in earlier onset of estrus expression and lower fertility. Delaying the administration of PGF to day 9 mitigated the earlier onset of estrus expression, however failed to restore pregnancy rates in cows receiving intravaginal devices with 0.5 g of P4.

1453 - EFFECT OF VITAMIN AND MINERAL SUPPLEMENTATION WITH THE ADDITION OF IODINE ON SERUM T3 AND T4 LEVELS OF HOLSTEIN COWS IN ANESTRUS

Author: Federica Ponte Ibarburu, Stephanie Lara, Luis Albornoz, Omar Bellenda

Objectives

The main objective of this study was to evaluate the effect of vitamin and mineral supplementation with the addition of sodium iodide on serum levels of T3 and T4 and on reproductive indicators in Holstein cows in anestrus.

Material and methods

The study was conducted in four dairy farms in Uruguay, between June to November 2022. Ninety anestrus Holstein cows were randomly assigned to one of the following 3 treatments: ***Control (C):*** no treatment was administered (n=29), ***Supplement (S)****:* treated with a commercially available vitamin and mineral complex (Vitmic Complex Cattle®, n=31), and ***Iodine Supplement (IS):*** treated with the same vitamin and mineral complex with added sodium iodine (n=30). Blood samples were collected from all the cows, before and 7 days after treatment administration. Serum was obtained by centrifugation at 3,000 rpm for 15 minutes and stored at -20°C. T3 and T4 serum levels were determined using commercial kits (CISBIO, France). Thyroid hormone levels and reproductive indicators were analyzed using ANOVA, utilizing the PROC GLM (SAS Studio®). The initial levels of both hormones (T3i and T4i) were considered as covariables in the statistic model. Treatment comparisons were analyzed by LSMEANS and were accepted as statistically significant differences when the P-value was less than 0.05.

Results

T3 and T4 levels remained within the normal ranges cited by Steinhoff et al. (2019) suggesting no subclinical hypothyroidism in the cows evaluated in this study. There were no significant differences between treatments in T3i and T4i ($P>0.05$); however, they influenced the statistical model as covariates ($P<0.0001$). The final T3 levels did not differ between treatments ($P>0.05$). Unexpectedly, the final T4 levels in the IS treatment were significantly lower compared to C treatment (33,8 vs 38, $P<0.05$). Additionally, it was observed, higher T3 and T4 initial and final levels in the primiparous group compared with multiparous ($P<0.05$). No significant differences were observed in the pregnancy rate (PR), intervals between calving and conception (ICC), and between estrus-to-ovulation (IEO), among treatments ($P>0.05$). However, the IS treatment exhibited the highest PR rate (77% IS vs 63%C, 52% S, $P=0.18$), shorter ICC (146 days in IS vs 206 days in C and 190 days in S, $P=0.32$) and IEO (56 days in IS vs 80 days in C and 95 days in S, $P=0.05$) compared to the other treatments, indicating a probable greater reproductive efficiency.

Conclusions

While the study presented unexpected outcomes regarding T4 levels, the positive impact on reproductive indicators in the iodine-supplemented group suggests potential benefits of sodium iodide supplementation for enhancing reproductive efficiency in anestrus Holstein cows. This research highlights the importance of nutritional interventions in influencing hormonal balance and reproductive function in dairy cows, suggesting avenues for further investigation into thyroid hormone interventions.

1470 - COMPARISON OF TWO COMPUTER-ASSISTED SEMEN ANALYSIS SYSTEMS IN THE EVALUATION OF BOVINE (Bos taurus taurus) SEMEN

Author: EMILIANO ALEJANDRO CORRALES-NAVARRO, DAVID URBÁN-DUARTE, HORACIO ÁLVAREZ-GALLARDO, JOSÉ ANTONIO AGUILAR-QUIÑONEZ, MIGUEL ÁNGEL GASTELUM-DELGADO, LEONEL AVENDAÑO-REYES

Objectives

The objective of this study was to compare two different computerized analysis systems for determination of sperm parameters in bovine semen.

Material and methods

Twenty-eight samples of bovine semen were analyzed using a commercial (SCA®) and a free-use (Sperm Motility Tracker) CASA software. Semen samples were collected and diluted in the same farm and transported to the Centro Nacional de Recursos Genéticos located in Tepatitlán, Jalisco, México, for evaluation. Videos of each semen sample were taken with a video camera spotted in a phase contrast microscope with a thermal plate (37° C); then videos were imported to SCA and SMT software to be analyzed. The response variables were: semen concentration, total cells detected, immotile, low, medium and rapid sperm, progressive sperm, curvilinear velocity (VCL), average path velocity (VAP), amplitude of lateral head-displacement (ALH), straight-line rectilinear velocity (STR), oscillation index (WOB), index of straightness, linearity index (LIN), beat cross frequency (BCF). Data were subjected to an analysis of variance under a completely randomized design, where the statistical model only included the fixed effect of type of system and the PROC GLM of the SAS statistical package (v. 9.4) was performed; significant differences were considered when $P \leq 0.05$.

Results

Both computerized systems resulted in similar results in the variables concentration, progressive, VCL, and VAP. However, the free-use system provided higher ($P<0.05$) values of total cells detected, immotile and rapid number of sperms. On the other hand, the commercial system was higher ($P \leq 0.01$) in low and medium number of sperms as well as in STR, LIN, WOB, ALH and BCF (Table 1).

Table 1. Results for response variables in each software.

Variable	Free Use	Commercial	S.E.	P-value
Concentration (M/mL)	58.24	55.53	8.15	0.8156
Total cells detected	322.11	178.65	40.65	0.0159
Number of sperms:				
Immotile	185.30	18.49	18.49	<.0001
Low	1.57	41.30	4.56	<.0001
Medium	5.92	60.03	8.45	<.0001
Rapid	131.03	37.46	21.45	0.0033
Progressive	91.50	97.50	18.96	0.8239
VCL (µm/s)	78.81	82.59	8.30	0.7488
VAP (µm/s)	51.74	47.69	5.15	0.5816

VSL (µm/s)	43.39	33.46	4.22	0.1028
STR (%)	30.67	62.37	2.42	<.0001
LIN (%)	21.16	37.90	2.09	<.0001
WOB (%)	25.14	56.31	2.01	<.0001
ALH (µm)	0.70	2.20	0.13	<.0001
BCF (Hz)	6.22	9.42	0.88	0.0134

Conclusions

The free-use and the commercial software showed similarities in some variables which can be used to determine these variables with a closer precision. However, the variation in the other kinetic variables can lead to a reading bias, which may generate problems when selecting sperm used for assisted reproduction techniques. For future research, it is recommended to consider that the microscope configuration can influence the reading of the programs, so it is necessary to establish a configuration that allows both software to provide more accurate analyses.

1480 - Evaluating the impact of sample-based pregnancy test procedures in milk production and daily cow time budgets of dairy cows

Author: Sushil Paudyal , Juan M Piñeiro, Douglas Duhatschek, Arthur Grando Pilati, Bhuwan Shrestha, Rajesh Neupane, Eun Ji Kim

Objectives
The objective was to evaluate the effects of milk sample-based pregnancy test (SBPT) procedures implemented on a commercial dairy farm on cow performance and behavior. The goal was to evaluate animal performance (milk production), daily time budgets (lying time, steps, lying bouts, lying bout duration), and reproduction parameters in cows tested for pregnancy through milk sample-based tests or transrectal ultrasound.
Material and methods
A total of 3 open dry-lot pens in a commercial dairy farm in North Texas were enrolled under repeated cross-over-design in treatment (TRT) and control groups (CON). The pens went through cross-over for 3 replications yielding a total of 6 individual treatment periods (n = 18 pens-period; 9 TRT pens and 9 CON pens). Cows in the treatment group were tested for pregnancy using IDEXX milk sample-based pregnancy testing kits in a milking parlor whereas, cows in the control group were tested for pregnancy using transrectal ultrasound at headlocks by a trained veterinarian. A leg-based accelerometer that recorded daily cow-level activity behavior was placed on the rear leg of a subgroup of cows. The statistical analysis was performed using the MIXED procedure in SAS with milk yield, lying time, step counts, and lying bouts as outcome variables and replications, crossovers, treatments, lactation, and DIM as the independent variables, and ID within the crossover periods was considered random effect within the model. Statistical significance was tested at $P<0.05$ and tendency was tested at $P<0.1$.
Results
Overall, cows in the CON groups were restrained in headlocks for an average of 1.7 hours per day whereas the TRT group was restrained for 0.6 hours on the day of pregnancy detection. The average parlor turnaround time in the TRT group was 14 minutes compared to 12 minutes in the CON group. The farm had an overall conception risk of 34% during the study period. Daily milk production was higher in the TRT group compared to the CON group by 1.6 (±0.46) lbs on the day after the pregnancy test ($P < 0.05$). The weekly total milk yield was also greater for the TRT group (51.47±0.49 vs 50.57 ± 0.48; $P<0.05$), indicating an overall positive effect of SBPT. The sampling procedure did not affect milk production on the day of the pregnancy test (50.69 ± 0.8 vs 50.33 ± 0.7 lbs; $p = 0.47$). On the day of testing, cows in the TRT group had a higher daily step count (1,839±129 vs 1,562 ±150, $P=0.02$) whereas the CON group tended to have fewer lying bouts (7.7±0.5 vs 8.3 ± 0.5, $P<0.1$). Lying bout duration was greater in the CON group (56.64+2.71 vs 50.35 + 2.87, $P=0.04$) minutes observed among the treatment groups. The daily total lying time of cows was not detected to be different with both groups lying 344± 31 and 347±32 ($P=0.8$) minutes per day respectively indicating that regardless of lockup time exposure, cows lay down a similar amount of time mostly due to compensatory effect on bout frequency and duration.
Conclusions
We conclude that SBPT procedures facilitate reducing the restraint time for cows managed in headlocks leading to improved milk production and dairy cattle time budgets, thus minimizing the management-induced stress in dairy cows.

1483 - Detection of estrogen and progesterone receptor expression in uterine biopsy specimens in instances of both normal and unsuccessful luteolysis in dairy cows.

Author: Michał Trela , Malgorzata Domino, Olga Witkowska-Piłaszewicz, Bartosz Pawliński

Objectives

During the ovarian cycle, estrogen and progesterone, potentially influenced by their endometrial receptors (estrogen receptor α and progesterone receptor, respectively), contribute to regulating the timing of both successful and unsuccessful luteolysis. This study sought to characterize the expression of estrogen and progesterone receptors (ER, PR) in the endometrium during dioestrus and anoestrus type III (luteal cyst) in dairy cows, offering a deeper understanding of their specific roles in the regulation of luteolysis.

Material and methods

Uterine biopsy specimens were obtained throughout the estrous cycles and anoestrus type III from 32 Holstein–Friesian non-lactating cows. Ultrasound examinations were conducted thrice (on days 9, 14, and 18 after ovulation) to confirm the presence of corpus luteum (CL) in 16 cows or follicular lutein cyst (FLC) in the remaining 16 cows. The collected samples were promptly frozen in liquid nitrogen and stored at –80°C for subsequent analysis. The localization of estrogen and progesterone receptors was determined using the method outlined by Robinson et al. (2001). ER and PR were visualized in fixed specimens through histological and immunofluorescent staining, employing light and confocal microscopy, respectively.

Results

The biopsy samples obtained during both progesterone states comprised luminal epithelium, superficial glands, deep glands, and subepithelial stroma. Regarding PR and ER, predominant immunostaining was observed (staining intensity/50 cells) in the nucleus of luminal epithelium (ER: 92 ± 108 / PR: 156 ± 19), gland tubule (ER: 97 ± 44 / PR: 111 ± 50), and gland opening cells (ER: 172 ± 32 / PR: 160 ± 39). Higher concentrations of estrogen receptor α were identified in the luminal epithelium compared to those in uterine stroma in both CL and FCL. Conversely, progesterone receptor concentrations were higher in bovine uterine stroma than in epithelial cells in both progesterone stages. Slight differences in progesterone receptor concentration in the luminal epithelium between CL and FLC were observed. No statistically significant differences ($P > 0.05$) were found in steroid receptor expression between CL and FLC in both progesterone states.

Conclusions

Our initial hypothesis, suggesting a significant difference in endometrial expression of ER and PR during two distinct progesterone states, was not substantiated. Nonetheless, notable distinctions were observed in the regulation of receptor expression among various types of endometrial cells. Previous studies have indicated that the concentration of progesterone receptor in the luminal epithelium peaks on days 6–8 and is typically undetectable at other stages of the estrous cycle, including, as per this study, anestrus type III.

1497 - Effect of body condition score, anestrus, and health status at the time of first-time fixed timed insemination on pregnancy rate in grazing lactating dairy cows

Author: German Dominguez, Santiago Corva, Rodolfo Luzbel de la Sota

Objectives

Evaluate the effect of body condition score (BCS), anestrus (ANE), and health status (HEA) at the time of first-time fixed time insemination (FTAI) on pregnancy rate in grazing lactating dairy cows.

Material and methods

An observational retrospective study was used, including a total of 19608 records of first time FTAI from cows calving from January 1st, 2016, to February 15th, 2023. Primiparous and multiparous Holstein cows with 50-63 days postpartum were included in this study. All cows were subjected to a FTAI protocol that included 2 mg of estradiol benzoate (EB), insertion of an intravaginal device containing 1.0 g of P4 (DIV) on day (d) 0; and administration of 150 µg PGF2α, 1 mg of estradiol cypionate (ECP), and 400 IU of equine chorionic gonadotropin (eCG) at the time of device removal on d7. All cows were tail painted at DIV removal to identify those in estrus after 48 h (YES=≥50% paint removed). Cows with paint removed at 48 h were FTAI, and cows without paint removed were administered 10 µg of GnRH and FTAI at 60 h. All AI were performed by farm personnel with frozen-thawed semen from a pool of sires. Pregnancy was diagnosed by ultrasonography at d30-43 after FTAI. The BCS was measured at the time of DIV insert (BCS1) and the time of pregnancy diagnosis (BCS2). The pregnancy risk to first FTAI was assessed by fitting a logistic regression model with lactation (LAC, 1 vs 2+), BCS (BCS1, <2.75 vs ≥2.75), anestrus (ANE; cycling [CYC], superficial anestrus [SA], deep anestrus [DA]), and health status (HEALTH; healthy [HEA], reproductive diseases [REP; retained fetal membranes, metritis, endometritis], non-reproductive diseases [NREP; mastitis, lameness], or both [BO]) as the main effects, and test day milk production (MILK) and days in milk (DIM) as a covariate (PROC GLIMMIX, SAS, 9.4).

Results

Primiparous cows had higher pregnancy rate (PR) compared to multiparous cows (39.7 vs 33.9%; Odds ratio [OR]-95%CI; 1.0 [LAC1, reference]; 0.74, 0.69-0.80; P<0.001), and cows with BCS≥2.75 had higher PR compared to cows with BCS<2.75 (37.0 vs 33.0%; 1.0 [BCS≥2.75]; 0.84, 0.78-0.91; P<0.001). Furthermore, diseased cows and cows in anestrus also had decreased pregnancy rates (P<0.001). Cows with no health events before FTAI had higher PR compared to cows with REP and NREP events (38.9, 36.0, 34.8, 32.3% respectively; OR-95%CI; 1.0 [HEA, reference]; 0.86, 0.80-0.93 [REP]; 0.86, 0.77-0.95 [NREP]; 0.74, 0.67-0.81 [BO]; P<0.0001). Similarly, cycling cows had higher PR compared to cows with SA, DA, and CYS (36.6, 34.4, 31.4% respectively; 1.0 [CYC, reference]; 0.90, 0.83-0.98 [SA]; 0.83, 0.71-0.98 [DA]; P<0.0001). MILK and DIM had no effect on PR (P>0.30).

Conclusions

The pregnancy rate was affected by lactation number, BCS, health status, and cycling status at the time of the first FTAI. This study was partially funded by UNLP-11V/272 to RLS.

1500 - Hormonal protocol based on injectable progesterone for estrous synchronization in sheep

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Objectives
The use of injectable progesterone (P4i) formulas has been tested in cattle as alternative treatments to intravaginal devices for estrous synchronization. The biological action of P4i needs to be elucidated to standardize treatments, including the administration route, dose, and frequency of application for estrus induction and/or synchronization protocols. Therefore, the aim was to determine the reproductive responses to hormonal protocols based on two P4i formulations for induction and/or synchronization of estrus in tropical hair ewes.
Material and methods
The study was carried out at the Unidade de Pesquisa Experimental em Caprinos e Ovinos of the Universidade Federal Fluminense, located in Cachoeiras de Macacu (22°S, 42°W), Rio de Janeiro, Brazil. All experimental ewes were previously evaluated by clinical and ultrasonographic exams. Only healthy animals with no reproductive or clinical abnormalities were selected. A total of 20 adult Santa Inês ewes (BCS and live weight: 2.8 ± 0.1; 48.1 ± 9.1 kg, mean ± SD) were allocated in an intensive system and fed with Capiaçu grass (*Pennisetum purpureum*, Schumach), 300 g of concentrate (18% crude protein and 75% total digestible nutrients) and free access to water and mineral salt. Ewes were divided into two experimental groups: control group (GCON; n = 10), which received an intravaginal device containing natural progesterone (0.36 g; Primer PR – União Química, São Paulo, Brazil) for eight days; treatment group (GP4i; n = 10), which received a long-acting P4i formula on D0 (75 mg; i.m.; Biorelease - Technologies LLC of Lexington, USA) and short-acting injectable progesterone (40 mg; i.m.; Progecio – 7% progesterone - União Química, São Paulo, Brazil) on the sixth daty. Both groups also received cloprostenol (120µg; i.m.; Estron, Agner União, São Paulo, Brazil) the eighth day. Sexual behavior and ovarian follicular population were monitored every 12 hours using active rams and transrectal ultrasound, respectivey. Blood was collected every 24 from the intravaginal implant insertion or administration of P4i for 12 days, and serum progesterone was measured using solid-phase radioimmunoassay. Data were analyzed using a mixed model, including the treatment, time and their interaction as main factors, and repetitions as a random factor. Data are presented as LSmean ± SEM. Ordinal variables were analyzed using Fisher's exact test. Differences were considered significant when $P < 0.05$ for all tests.
Results
The interval from cloprostenol administration to the estrus onset tended to be longer in the GP4i than in the GCON(68.5 ± 5.9 h vs 52.2 ± 5.4 h, respectively; $P = 0.06$). There were no differences between groups (estrous length: 36.7 ± 5.1 h vs 42.6 ± 5.3 h; ovulation rate: 80% vs 90%; interval from cloprostenol administration to ovulation:106.6 ± 4.6 h vs 99.6 ± 8.5 h; diameter of the largest follicle: 6.0 ± 0.5 mm vs 6.2 ± 0.2 mm for GP4i and GCON, respectively). Progesterone concentrations varied along time ($P < 0.0001$), with no treatment effect, and a tendency for an interaction between treatment

and time (P= 0.08). The highest P4 concentrations were obtained in the first sample after its administration (17.5 ± 1.3 ng/mL), with the values declining over time. During all time evaluated, including the last day, circulating P4 values were above 1 ng/mL.

Conclusions

The combination of P4i tended to increase the estrous length without other effects. The treatment allowed to maintain circulating P4 values similar to those provided by intravaginal devices.

1512 - Investigation of the vaginal microbiota of dairy cows through genetic sequencing of short (Illumina) and long (PacBio) reads and associations with gestational status .

Author: Anne Souza, Amanda Zamgirolamo, Marcio Costa, Marcelo Seneda, AMAURI ALFIERI

Objectives

Next-generation sequencing (NGS) has recently become a standard method for studying the microbiota in humans and animals. Although most studies have focused on intestinal. microbiota, vaginal bacteria have been shown to play important roles in immune regulation and protection against vaginosis. After the development of NGS, unprecedented information regarding the profile of bacterial communities in the bovine reproductive tract related to health and reproductive diseases has been obtained. Illumina technology is responsible for generating >90% of the known sequencing data and has been considered the standard because of its high reliability and low cost. This technology minimizes the time and cost of sequencing by performing short reads (75–600 base pairs at a time), covering only small and specific regions of the 16S rRNA gene, which is most often used for taxonomic classification. Thus, this technology does not allow for classification at the species level. Conversely, NGS platforms with the capacity to read long DNA sequences can sequence the full-length 16S rRNA gene (1500 bp), providing accurate identification at the species level. Long-reading NGS platforms have contributed to the study of microbial diversity from a deeper perspective and evaluating the agreement between the results of both technologies is necessary for better interpretation of results obtained from Illumina studies. The PacBio platform (Pacific Biosciences) is a technology that produces high-fidelity reads of complete 16S rRNA genes, improves the sensitivity and specificity of the taxonomic profile, and reduces the risk of misclassified reads. To date, no study has used long-read sequencing to characterize the vaginal microbiota of cows.

Currently, microbiota studies emphasize the pathogenicity of bacteria; however, by understanding the interaction between the host environment and its microorganisms in greater depth, it is possible to identify biological markers that are beneficial to the host. Therefore, the objective of this study was to characterize the bovine vaginal microbiota at the species level using long-read sequencing (PacBio) and to compare it with results of short-read sequencing (Illumina).

Material and methods

Thirteen Holstein Friesian cows with a mean age of 5 ± 1.3 years housed at a dairy farm were evaluated. The selected cows were multiparous, and were not subjected to a hormonal protocol for synchronization and ovulation induction. On the day of estrus, the cows were inseminated following reproductive management adopted on the farm. Pregnancy was diagnosed 30 d after insemination using ultrasonography and the cows were classified as pregnant or non-pregnant. A vaginal sample from each cow was collected before AI using a sterile swab introduced into the caudal portion of the vagina. For the DNA extraction, a commercial kit was used, following the manufacturer's specifications. The extracted DNA was subjected to two different analyses: the first using short reads (Illumina) and the second using long reads (PacBio). For short-read sequencing, the V4 region of the bacterial 16S rRNA gene was amplified using

polymerase chain reaction (PCR) for sequencing using Illumina. The full-length 16S rRNA gene was amplified using PCR. For the comparison between methods (Illumina versus PacBio), the same parameters used for short-read analysis were used in mothur, except for the length of the reads that were set to a minimum of 1500 bp. Alpha and beta diversity was calculated.

Results

PacBio sequencing yielded 366,509 reads that were assigned to 476 species from 27 phyla. However, none of the most abundant reads (>1%) could be classified at the species level. Illumina sequencing yielded more reads and consequently was able to detect a more observed species, but PacBio sequencing was able to detect more unique and rare species.

Conclusions

The composition of the vaginal microbiota varies according to the sequencing method used, which might complicate the interpretation of results obtained in the majority of the current studies. The present study expands on the current knowledge of bovine microbiota, highlighting the need for further efforts to improve the current databanks.

Financial support: National Institute of Science and Technology for Dairy Production Chain (INCT–LEITE), CNPq, CAPES, and Fundação Araucária (FAP/PR).

1546 - Injectable mineral supplementation influences ability of Anti-Müllerian hormone as biomarker for reproductive traits in Holstein cows exposed to severe heat stress

Author: Luis Armando Contreras Méndez, Pablo Luna Nevarez, Pedro Alan López Castro, Guillermo Luna Nevarez, Juan F. Medrano, Milton G. Thomas, Hamad M. Saad, R. Mark Enns, Scott E. Speidel

Objectives

Reproductive management in cows during summer in Northwest Mexico is challenging for dairy producers due to harsh weather conditions. Identification of novel endocrine markers for fertility may provide a useful strategy to improve cow reproduction. The Anti-Müllerian hormone (AMH) has been recognized as a potential biomarker for reproductive traits in dairy cows; however, its predictive ability appears to be weakened by hot and humid climatic conditions. It has been reported that supplementing with minerals can help reduce the negative effects of heat stress on cattle fertility. Therefore, our objective was to evaluate the influence of injectable mineral supplementation with FOSFOSAN™ (P, Mg, Se, Cu and K. Virbac Laboratories) on the ability of serum AMH concentration as an endocrine marker for fertility traits in dairy cows exposed to severe heat stress.

Material and methods

During late summer month, one hundred and twelve Holstein cows were randomly assigned to one of two treatments, a control (CON; n=62) and a mineral supplemented group (SUP; n=50). Cows were selected from a Holstein dairy farm located in a hot and semidesert region. Ambient data were collected from a nearby climatic station and used to calculate the temperature-humidity index (THI). The THI averaged 82.4 units throughout the study suggesting environmental conditions indicative of severe heat stress. All cows received a hormonal treatment to synchronize ovulation (CIDR-Synch) followed by a fixed-time artificial insemination (FTAI). At the initiation of the CIDR-Synch protocol, a blood sample was collected and used to determine serum AMH concentrations. Rectal ultrasonography evaluation was performed to measure dominant follicle (DF) diameter three days before FTAI, as well as Corpus Luteum (CL) size the days 6, 12 and 18 after FTAI. Ultrasound examination was also used for pregnancy diagnosis 30 d after FTAI, which was used to calculate conception rate (CR). Rectal temperature (RT) and respiratory rate (RR) were collected bi-weekly during the study. A mixed effects statistical model including treatment and time as fixed effects was used to compare average values for serum AMH, ovarian traits and physiological variables between treatments. Diameters of the dominant FOL and CL were analyzed using a completely randomized block design with repeated measures over time. A “Chi-square” test was performed to compare CR between SUP and CON treatments. Finally, correlation and regression analyses were used to evaluate the relationship between AMH and ovarian traits within treatments.

Results

Serum concentrations of AMH were higher in SUP group compared to CON group (429.01 ± 46.79 vs. 135.41 ± 10.17 ng/mL; $P<0.01$). Similarly, higher values were observed in DF (12.97 ± 0.36 vs. 10.05 ± 0.48 mm) and CL (2.58 ± 0.06 vs. 1.31 ± 0.07 cm) diameters in SUP group compared to CON group ($P<0.05$). In addition, a trend to reduce

both RT and RR was detected in the mineral-supplemented group (P=0.086). A more detailed analysis of the progress in ovarian activity detected that mineral supplementation increased DF growth across time periods (P<0.05) and tendency was observed for CL growth (P<0.10). However, slight, non-significant metrics were observed between the two groups for growth of DF and CL. Conception rate was higher (P<0.05) in SUP group compared to CON group (44.0 vs. 32.2 %). A moderate correlation was observed between serum AMH with DF (r=0.46; P<0.01) and CL (r=0.41; P<0.05) diameters in SUP group, whereas no associations were observed between AMH and ovarian structures within CON group. Serum AMH concentrations appeared to be a predictor of measures of DF (R^2=0.21; P<0.01) and CL (R^2=0.17; P<0.05) diameters but only in Holstein cows exposed to severe HS that received a supplement with minerals.

Conclusions

In conclusion, injectable mineral supplementation with FOSFOSAN™ under severe heat stress appeared to enhance serum AMH secretion in Holstein cows subjected to an estrous synchronization program and FTAI. The improved AMH concentration appeared to have helped maintain the growth of both dominant follicles and CLs, which led to increase CR. In addition, supplementation with FOSFOSAN™ in Holstein cows exposed to severe heat stress suggested AMH concentrations as potential predictive biomarker for fertility.

1563 - THE ADDITION OF AN ANTIOXIDANT IN THE CRYOPRESERVATION PROCESS WILL IMPROVE THE PRESENCE OF THE ACROSOME IN BOVINE SPERMATOZOA

Author: ITZAYANA MEJIA FLORES, JAVIER HERNÁNDEZ IGNACIO, NATALIA CHIQUETE FÉLIX, MONICA GABRIELA NAVARRETE GARCÍA, SANDRA HERNÁNDEZ GARDUÑO

Objectives

The freezing and thawing process causes oxidative and osmotic stress, heat shock, intracellular ice formation, changes in the lipid and protein composition of the plasma membrane, decreased sperm viability and motility, damage to the acrosome, tail and mitochondria. In addition, it induces the reorganization of lipid membranes, resulting in increased membrane fluidity and intracellular calcium, leading to a process called cryocapacitation. The premature induction of capacitation and acrosomal reaction alters mitochondrial function and thus reduces sperm motility. The mitochondrion is the energy source of respiration and also the main site of reactive oxygen species (ROS) generation. ROS have the ability to reversibly or irreversibly alter cellular function. It has been proposed that ROS modify sperm biochemistry and physiology. On the other hand, antioxidants may protect spermatozoa from damage produced by ROS. There are few studies on the effects of a supplemental antioxidant targeting mitochondria in semen cryopreservation, and no report has evaluated the protective effects on sperm quality parameters during bovine semen cryopreservation. The objective of the present study was to evaluate sperm viability and membrane and acrosome integrity upon thawing by eosin-nigrosin and hypoosmotic Coomassie blue (HOST/Coomassie) staining of bovine spermatozoa cryopreserved in commercial medium [AndroMed®] with different concentrations of an antioxidant targeting mitochondria [MitoTEMPO®].

Material and methods

Three healthy Brangus bulls were used, from which three ejaculate samples were obtained and evaluated for each bull, the ejaculates were divided for freezing in 3 groups: Group 1 control, Group 2 with 25 mM and Group 3 with 50 mM, the different concentrations of the antioxidant were added at the moment of strawing. After thawing, the evaluation of motility, eosin-nigrosin and HOST/Coomassie staining of bovine spermatozoa was performed. One-way ANOVA (Tukey's multiple comparison test) was used to compare the means of motility, live vs. dead, membrane functionality and acrosome integrity in spermatozoa between treatments, using GraphPad Prism Version 5 (GraphPad Software, Inc., La Jolla, CA, USA) at P value less than or equal to 0.05.

Results

The results obtained show an improvement in motility in the cryopreserved spermatozoa with the 50 mM concentration compared to those with 25 mM of the antioxidant (81.6% vs. 76.6%). As for sperm viability and acrosomal integrity, a greater positive response to the HOST test was observed in group 2: 95.92% compared to group 1: 94.33% and group 2: 92.71%. The presence or absence of the acrosome in the spermatozoa was evaluated with Coomassie blue staining, the group added with 25 mM of the antioxidant had a marked increase in the percentage of spermatozoa with intact acrosome 58.88% in comparison with the control group 36.21% and the group with 50 mM of the antioxidant 36.34%. There was no statistical difference between the three

groups in the results by eosin-nigrosin staining, showing similar percentages of live spermatozoa in group 1 with 56.46%, 60.53% in group 2 and 59.57% in group 3.

Conclusions

During the freeze-thaw process, the excess of ROS produced due to oxidative stress causes lipid peroxidation, causing an attack on polyunsaturated fatty acids, resulting in structural and functional damage to the plasma membrane of the spermatozoa, these damages conjugated with the by-products formed cause changes like cryocapacitation and loss of acrosome integrity. The incorporation of antioxidants directed to the mitochondria can prevent membrane damage, an action that we can verify in our results where the mean percentage of intact spermatozoa in the membrane was significantly higher in the group added with 25mM of the antioxidant, preventing oxidative damage due to freezing and thawing stress. These results support the importance of using antioxidants during sperm cryopreservation, which will be very beneficial for AI in terms of beef cattle production.

1571 - Evaluation of the Quality, Quantity, and Viability of Cumulus Oocyte Complexes Recovered from Ovaries of Creole Slaughter Cows Using Different Transport Media

Author: Rocío Silvia Sandoval Monzón, Luis Felipe Ruiz García, Medalith Sierra Marquina

Objectives

To evaluate the quality, quantity, and viability of cumulus oocyte complexes recovered from ovaries of Creole slaughter cows using different transport media

Material and methods

This study was conducted at the Faculty of Veterinary Medicine of UNMSM. In the experimental material acquisition phase, 50 ovaries from Creole cows were evaluated. These ovaries were subjected to different transport media to analyze the quality, quantity, and viability of cumulus oocyte complexes. The experimental design included the use of five transport media: distilled water, PBS (Phosphate Buffered Saline), 0.9% NaCl, Tris enrichment medium, and no medium (Barberino et al., 2018). Each medium was applied to 10 ovaries, and the quantity, quality, and viability of the obtained cumulus oocyte complexes were assessed. The transportation and storage time was 16 hours after ovary retrieval. In the experimental procedure, ovaries were randomly divided into the five experimental groups at the slaughterhouse and transported at 4°C using different transport media. The Slicing technique (Rodríguez, 2013) was employed for the recovery of cumulus oocyte complexes, followed by searching, classification (good quality: I and II), and measurement of cumulus oocyte complexes using specialized software. Cumulus oocyte complexes were stained with trypan blue, and viability was assessed according to established protocols. Data analysis was carried out using statistical tests such as Chi-square and Friedman, using IBM SPSS version 25.0. The independent variables were the transport media, while the dependent variables were the quality, quantity, and viability of cumulus oocyte complexes. A significance level of 5% was considered.

Results

Finding a transport solution for ovarian tissue involves controlling various factors, including toxicity, nutrients, and harmful compounds that affect follicle health during transportation. Additionally, cost, availability, and the time required to transport ovaries to the laboratory must be considered (Barberino et al., 2018). The results indicate that there was no difference in the quantity of cumulus oocyte complexes from cattle recovered using Tris enrichment medium (18 cumulus oocyte complexes/ovary), physiological saline solution (19.6 cumulus oocyte complexes/ovary), and the no-medium group (18.8 cumulus oocyte complexes/ovary). However, a lower quantity of cumulus oocyte complexes was recovered using PBS medium (15.6 cumulus oocyte complexes/ovary) and distilled water medium (16.3 cumulus oocyte complexes/ovary). Regarding the quality of cumulus oocyte complexes, the Tris treatment and physiological saline treatment led in the quantity of cumulus oocyte complexes recovered of good quality (13.7 and 12.9, respectively). This was higher than the quantity of cumulus oocyte complexes recovered of good quality in the other treatments. Similarly, the Tris and physiological saline treatments had a higher quantity of recovered viable cumulus oocyte complexes (14.2 and 13.9, respectively), along with the no-transport medium

group (13.5 viable cumulus oocyte complexes/ovary). However, when evaluating the percentage of good-quality cumulus oocyte complexes and the percentage of viable cumulus oocyte complexes, it was found that the Tris group had the highest results (76.1% and 78.9%, respectively), significantly superior to the percentages of the other groups. This is because the Tris enrichment medium has appropriate osmolarity and concentrations of amino acids, sugars, vitamins, minerals, and antioxidants that can reduce oxidative stress and DNA damage in different cell types.

Conclusions

Conclusions:

The results highlight that the Tris enrichment medium was consistently superior in terms of quantity, quality, and viability of cumulus oocyte complexes in cattle, suggesting its use to optimize bovine reproduction practices.

References:

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1585 - First report of Schistosomus reflexus and thoracophagus conjoined twins in Luis Moya, Zacatecas, México.

Author: Guadalupe Geraldine Pliego Moreno

Objectives

The objective of this case report is to describe the clinical and pathological aspects observed in Holstein foetuse

Material and methods

Three Holstein foetuses was stillborn by c-section, in Luis Moya, Zacatecas, México in 2019. Two of them were joined by the thorax. They were the result of an artificial insemination. No information was available regarding the breeding history. Due to the special circumstances of birth, only a limited physical examination was performed. The body was carefully dissected and photographic records were made of all recognizable anomalies.

Results

Case 1: The removed foetus was a dead, malformed, monster calf with undefined sex. It was found with visceral eventration, characterized by incomplete closure of the ventral body, the absence of thoracic and abdominopelvic walls along with exposed heart and lungs and underdeveloped and exposed gastrointestinal system; in addition, ankylosis of all the four limbs, positioning near the skull, shortened and inverted vertebral column, severe dorsiflexion, tail and sacrum close to the head, and protruded tongue were noted. Based on these findings, it was diagnosed as a case of *Schistosomus Reflexus*.

Case 2: The calves were classified thoracophagus conjoined twins. It had two heads and two bodies that converged on a large thorax and umbilical cord. The animals had four limbs, each were positioned normally, each one has its vertebral column, both female. Each one had a pair of lungs separated by the pleura, the lungs of the left calf were found collapsed and with marked congestion, in addition to multi focal whitish areas; those of her twin were found healthy. Both shared a heart, which presented cardiomegaly, measuring 16 cm height and 13.6 cm width and defect in the interventricular septum. The mouth, pharynx, and oesophagus of each animal appeared normal. Each stomach had a rumen, reticulum, omasum and abomasum complete relative to the age of the animal.

Conclusions

This is the first reported case of *schistosomus reflexus* foetus and thoracopagus conjoined twins in the Luis Moya, Zacatecas area. Dystocia of the dam is commonly seen due to foetal monstrosity with abnormal skeleton, spine, and voluminous exposed viscera. Congenital anomalies are found in almost all breeds of cattle and considered to be important because the loss of offspring and overall management cause huge economic losses. Various types of foetal malformations are associated with dystocia in cattle. Identification of a aetiology of the developmental anomalies is often extremely difficult for several reasons, defective development alone often does not give clues to the specific cause, teratogens such as viruses, plants and toxins cannot be demonstrated at the time of expulsion of the defective foetus or even after intensive pathological and toxicological investigations. Calves are occasionally found with *schistosomus reflexus* due to genetic abnormalities that are most probably because of autosomal recessive

genes that are considered to affect the early post-gastrulation embryo involving the intermediate mesoderm. Therefore, healthy cows, bulls, and bull-semen (used in artificial insemination) with a good pedigree should be considered for breeding purposes to avoid this hereditary defect. Conjoined twins occur in virtually all animal species, including humans. Among domestic species, cattle have the highest incidence of congenital duplication anomalies. Two hypothesis have been proposed and disputed: fusion and fission. The fusion hypotheses develop from the idea of a secondary fusion between two separate embryonic axes. Fusion occurs after the fate of the cells has been determined. The fission hypothesis involves the incomplete separation of the inner cell mass of the embryo, with variable degrees of disjunction of the embryonic axes.

The lesions found in the lungs of one of the foetus could be related to the position in the uterus and meconium aspiration, however it is a curious finding. The presence of cardiomegaly may be associated with the fact both calves shared a heart.

This cases of teratology have been unique events and have not occurred again in this place.

1610 - Effect of the design and type of device with progesterone for synchronizing ovulation on the habituation time and behavioral response of hybrid heifers.

Author: FELIPE MONTIEL PALACIOS

Objectives

The objective of the present work was to determine if the design and type of device with progesterone for synchronizing ovulation affects the habituation time and behavioral response of hybrid heifers.

Material and methods

Material and methodsThe procedures that will be carried out during this experiment will adhere to the Official Mexican Standard, published in the Official Gazette of the Federation on the technical specifications for the production, care and use of laboratory and experimental animals. As well as the internal regulations of the UV faculty of veterinary medicine and zootechnics. Title 7.Study areaThe study was carried out in a livestock production unit (UPP), which is dedicated to the production of calves for slaughter, located in Macuspana, Tabasco, Mexico, located at 17°45'17" N and 92°33'32" W , at a height of 10 meters above sea level, with a tropical climate, temperature and average annual precipitation of 26.4 °C and 3,186 mm, respectively, according to data from the National Institute of Statistics and Geography.

Characteristics of the experimental unit100/300 Simbrah hybrid pubertal heifers were selected with average age (4-6 years), days postpartum (60-90 days), live weight (350-450kg) and body condition of 5 to 7 (scale 1 to 9).

Hormonal treatments to synchronize ovulation.100 hybrid heifers were randomly selected and divided into ten groups (n=10), to assign one of the different conventional hormonal treatments for synchronizing ovulation with fixed-time artificial insemination (FTAI). Treatments, day (0) an intravaginal device was placed with different concentrations of natural progesterone (DV+P)(T1 = 0.6 g, T2 = 1.0 g, T3 = 0.6 g, T4 = 1.2 g, T5 = 1.9 g, T6 = 1.3 g, T7 = .750 g, T8 = 1.0 g, T9 = 0.5 g and T10 = CONTROL) and 2 mg of estradiol benzoate (BE) was applied. Day (8) the intravaginal devices were removed, 400 IU of equine chorionic gonadotropin (eCG) and a dose of prostaglandin (PGF2α) were applied according to the recommendations of each device. Day (10) fixed-time artificial insemination (FTAI) was performed corresponding to 48-56 h after removal of the devices. The control group only received a placebo at the same time as the treatments described above.

Post-synchronization ethology.Observation of behavior after insertion of the vaginal devices was carried out in all hybrid heifers in the management pen and in the pasture. A plan of the management pen was made to carry out behavioral observations of the synchronized heifers and descriptions of each area. The habituation time to the device was also evaluated through direct observations supported by video recordings. The sampling and recording method used is continuous focal respectively. The observation was carried out from the beginning of the placement of the vaginal devices until day 3 and on day 8, which was the removal of the DVs, at intervals of every 2 hours for 30 minutes, at the hours of 10:00, 12:00, 14:00 and 16:00 hours.

Variables and statistical analysis.The variables considered for analysis were habituation time and behavioral response through Xi2 available in the SAS statistical package.

Results

The behaviors that occurred most frequently were slow flight, stopping, flagged tail, and arched tail with 56, 66, 50 and 56%, respectively. The average waiting time in the press was 6.15 min and the escape time after synchronization was 22.1 sec, for all synchronized females regardless of treatment.

Conclusions

It is concluded that the different designs and types of devices impregnated with natural progesterone for ovulation synchronization affected the habituation time and behavioral response of hybrid heifers.

1616 - Pregnancy rate in bovine females transferred at a fixed time with embryos produced in vitro and fresh under tropical conditions

Author: FELIPE MONTIEL PALACIOS

Objectives

The objective was to determine the pregnancy rate in bovine females transferred at a fixed time with embryos produced in vitro and fresh under tropical conditions.

Material and methods

Materials and methods

The study was approved by the Bioethics and Animal Welfare Commission in accordance with the Internal Regulations of the Faculty of Veterinary Medicine and Zootechnics of the Universidad Veracruzana (Title VII chapters I, II, III, IV, articles 92 to 124) and comply with the provisions of NOM-033-ZOO-1995, section 6.1.b.

Laboratory Location and study site

The production of in vitro embryos was carried out at the RGA IN VITRO Laboratory, in Boca del Río, Veracruz. The first place was with cows from which the oocytes were obtained for the in vitro production of embryos belonging to a commercial dairy herd (Rancho Fuentezuelas) located in Tequisquiapan, Qro. The second place was with gray Brahman donors (Rancho El Brinco) located in the town of El Brinco, Ver. The transfer of embryos produced in vitro was carried out in dual-purpose cattle ranches located in the municipalities of Paso del Macho, Veracruz, Atoyac de Álvarez and Florencio Villareal, Guerrero.

Experimental design and Production of bovine embryos

Treatment 1 = 16 embryos of the Bos indicus race and Treatment 2 = 67 embryos of the Bos taurus x Bos indicus race grade 1 (excellent) produced in vitro, were transferred fresh (n=67). Oocytes obtained by ultrasound-guided follicular aspiration from Holstein and Brahman donors. After searching and selecting oocytes, they were stored in microtubes (Microtubes for Cell Culture) with maturation medium, and were transported to the RGA laboratory, approximately 8 h of road travel, in a portable three-gas (5% O2, 5% CO2 and 90% N2). Fertilization was carried out with thawed semen from Gyr and Brahman breed bulls.

Selection and management of recipients

The 83 embryos were transferred to the same number of recipients. The health management of the recipients was carried out through vaccination against clostridiasis and pastereulosis, bovine viral diarrhea, infectious bovine rhinotracheitis, parainfluenza type 3, and bovine paralytic rabies; They also received treatment against internal and external parasites.

Ovulation timing program

Ovulation was synchronized using an Intravaginal Device with 1.3 g of natural progesterone plus the application of 2 mg of EB via IM on day 0. On Day 5, 400 IU of eCG IM plus 500 mg of cloprostenol sodium IM were applied. On Day 8, the vaginal device was removed and the IV was applied 1 mg estradiol cypionate IM.

Embryo transfer and pregnancy diagnosis

The transfer of the embryos was performed by the same veterinarian in all the recipients who, upon transrectal palpation, presented a well-implanted CL with a diameter ≥ 1.5 cm. The transfer applicator was covered with a sterile cover, introduced into the vagina, passed through the cervical canal by transrectal manipulation and directed towards the uterine horn with corpus luteum, where the contents of the straw were deposited in the third medium. Each recipient received one embryo.

Pregnancy diagnosis

Pregnancy diagnosis was made by transrectal ultrasonography with 3.5 MHZ convex transducer, 60 d after transfer.

Statistic analysis

A mixed threshold model with PROC GLIMMIX (SAS, 2014) was used for the pregnancy rate analyses. Conditional on the fixed and random effects, the pregnancy rate was assumed to follow a Bernoulli distribution.

Results

Table 1 shows the effect of the synchronization protocol on the inclusion rate by treatment.

Table 1. Effect of the synchronization protocol on the inclusion rate by treatment

Location	Synchronized	Transferred (%)
Atoyac	60	31 (51.6)
Cruz Grande	60	36 (60)
Paso del Macho	34	16 (47)
Total	154	83 (54)

Table 2 shows the effect of geographic region on the pregnancy rate.

Table 2. Effect of geographic region on pregnancy rate

Location	Transferred	Pregnants (%) / IC (95%)
Atoyac	31	13 (42) / (0.38 ; 0.65)[a]
Cruz Grande	36	19 (53) / (0.28 ; 0.55)[a]
Paso del Macho	16	08 (50) / (0.27 ; 0.54)[a]
Total	83	40 (48)

[a] There was no statistical difference

Conclusions

The pregnancy rate in bovine females transferred at a fixed time with embryos produced in vitro and fresh under tropical conditions was acceptable.

1627 - A case of white heifer disease in Polish Holstein-Friesian cattle

Author: Aleksander Butkiewicz, Bartłomiej M. Jaśkowski, Michał Barczykowski, Zbigniew Lach, Maciej Zdun, Kacper Żukowski, Jędrzej M. Jaśkowski

Objectives

White heifer disease is a genetic disorder recorded mainly in white-coated cattle breeds. Most often, single cases of this defect were mentioned, but it may affect a larger number of females. It is accompanied by partial genital aplasia and reduced fertility. Below is a case of white heifer disease in a Polish Holstein-Friesian heifer (PL005458128986) from the Breeding Center. Heifer was born on May 3, 2022. Her father was the bull ALUM (US-3147853565), and her mother – Helga 53 (PL005520529369). The heifer had not previously shown any disturbing signs that would have attracted the staff's attention. At approximately 12-13 months of age, the heifer began to go into heat at irregular intervals. After several unsuccessful insemination attempts, the inseminator decided to call a veterinarian to conduct a detailed examination of her reproductive system.

Material and methods

The veterinarian taking care of the herd performed a clinical examination and took ultrasound screenshots. During clinical rectal examination, the uterus (its right horn) was threadlike, and the presence of the second horn was not detected. The boundary between the cervix and the existing uterine horn was not palpable. Adjacent to the uterine horn was a natural-sized ovary, which on the day of the examination revealed the presence of a well-developed corpus luteum, a diameter of 16 mm. Despite these abnormalities, it was decided to administer an analog of prostaglandin 2 alpha, after which the heifer showed estrous symptoms.
The heifer's body condition score (BCS) was assessed and morphometric measurements of the heifer were performed. Then an ear sample was taken for genomic testing, blood for RNA isolation, and a hair sample. Additionally, photos were taken using an iPhone 13 mini (Apple, Cupertino, CA, USA).

Results

The coat was black and white with the predominance of black. The heifer's BCS on the day of examination was 3.5, her height at the withers was 137 cm, the distance from the center of the anus to the base of the clitoris: 115.44 mm, and the distance from the center of the anus to the dorsal commissure of the labia: 70.47 mm, so the length of the labia was slightly above 44 cm. At the same time, in the lower symphysis of the labia, a brush of relatively long hair was noticed, resembling that observed in heifers affected by frimartinism. Moreover, tests of genetic characteristics performed at the National Research Institute of Animal Production showed the absence of genetic defects such as BLAD, DUMPS, and other routinely assessed defects associated with reduced fertility (HH1, HH3, HH4, HH5, HH6, HH6, CDH).

Conclusions

The presented case is the first of this type described in elite Holstein-Friesian cattle in Poland. In the next steps, genetic tests are planned in the context of further diagnostics and identification of mutations. These studies may contribute to the earlier detection of animals with this defect and, consequently, by placing appropriate probes on SNP microarrays, screening for developmental abnormalities in the cattle population.

1628 - Is the barium chloride test a reliable method for detecting pregnancy in cows?

Author: Gracjan Wozniak, Bartłomiej M. Jaśkowski, Sandra Kunda, Jakub Kulus, Jędrzej M. Jaśkowski

Objectives
Detection of barium chloride in urine is a chemical method of pregnancy examination in cows as well as goats, buffaloes, sheep, pigs, camels, and alpacas. This method has been known for decades. Recently, however, interest in it has been increasing again. The test involves a unique reaction of urine with barium chloride, which is triggered only in the presence of a fetus or embryo. The principle of the test is that progesterone present in urine during pregnancy prevents the sedimentation of barium chloride, while estrogen promotes it. Thus, in non-pregnant cows, white sediment forms in the urine, while in pregnant cows, the urine remains clear. The study aimed to assess the usefulness of the barium chloride test for testing pregnancy in cows.
Material and methods
A total of 53 cows were examined, on average 63 days of pregnancy (23-256 days after insemination). Pregnancy recognition was based on the ultrasound examination with the use of Dramiński iSkan equipped with a 7.5 MHz probe. Urine collected during spontaneous urination or obtained using a catheter directly from the urinary bladder was collected from all examined animals. The tests used 0.5 ml of urine to which a 0.5 ml of 12% aqueous solution of barium chloride was added. The test readings were made on a base of previous studies.
Results
The percentage of concordant results to the ultrasound examination (positive and negative) was 43.6%. From this group, for 6 cows the barium chloride test was considered questionable. Among pregnant cows, 52.4% of the results were consistent with the ultrasound examination (after excluding questionable results). Of the 18 non-pregnant cows, the results consistent with the ultrasound examination were obtained in 46.1% of the cows.
Conclusions
The results were not satisfactory. Perhaps the low reliability could be the effect of the concentration of the solution used in the tests, a small amount of urine used, a large amount of barium chloride solution added, or the different capacities of the test tubes. In previous studies, the accuracy of the test was estimated at 70-95% from 15 to 210 days of pregnancy. The sensitivity and specificity of the test were 54% and 85%, respectively. In research conducted in Nepal, the accuracy was 69.5% in the case of confirming pregnancy and 100% for non-pregnant cows. Overall, the value of the test is legitimately controversial. At the current stage of method development, the test is unable to compete with PAG assays. However, it seems to have some perspective, primarily as it is feasible in all barn conditions (periodic PAG determination) in all categories of animals (heifers, cows), is cheap, and does not require special equipment (such as an ultrasound) and high-class specialists. This test – if the spontaneous urine is collected – also meets high requirements to ensure maximum welfare of the tested animals. However, if the urine is collected with a catheter, its non-invasiveness is at least questionable. Taking into account the relatively long time required to collect and process samples, it is difficult to consider the above-mentioned test as "fast".

1634 - Corpus luteum in embryo recipient heifers – practical aspects

Author: Bartłomiej M. Jaśkowski, Bartosz Czech, Jędrzej M. Jaśkowski

Objectives

Synchronization to induce ovulation at a specific time has been widely used in both cattle production and research for many years. However, despite the increasingly complex synchronization programs, the effectiveness of conception after their application has been slowly declining in recent years. Progressive technological development has introduced new possibilities to animal production, such as the production of *in vitro* embryos or their transfer after superovulation. While, at the time of insemination/natural mating, it is not possible to clearly assess the potential of the future corpus luteum, when using assisted reproductive techniques such as embryo transfer, the assessment of the corpus luteum before transferring the embryo may translate into success. Therefore, the aim of the study was to indicate the size parameters of corpora lutea of heifers after synchronization and to link them with the occurrence of pregnancy.

Material and methods

The study used the results from 475 heifers – embryo recipients, to whom a fresh embryo was transferred. The embryo transfer was preceded by synchronization according to the same scheme: 2x 0.5 mg cloprostenol IM (Estrumate, Intervet International) 14 days apart. In all females, the day of estrus symptoms was recorded. The embryos were transferred on the 7th day after the first symptoms. Immediately before the procedure, the recipients underwent an ultrasound examination using a device (iScan, Dramiński, Poland) equipped with a linear transducer with a frequency of 7.5 MHz. The images of the ovaries were recorded in the device's memory, then the surface area of the corpus luteum in its widest section and its volume were measured using the ultrasound software. In the case of a cavity inside the corpus luteum, its parameters were subtracted from the parameters of the corpus luteum itself, so that only the surface area and volume of the luteal tissue were assessed each time. Statistical analysis was performed based on Pearson's χ^2 test of independence and the receiver operating characteristic (ROC) curve with Youden's J statistic, followed by logistic regression.

Results

Pregnancy was detected in a total of 40.2% of females (191/475), of which more were found in heifers with a cavitary corpus luteum on the day of transfer – 45.8% (93/203; $P<0.05$) – compared to females with a homogenous corpus luteum – 36% (98/272). The mean area of the corpus luteum at its widest section was 3.17±1.15 cm^2 (3.16 cm^2 range 0.95-6.83 cm^2 and 3.17 cm^2, range 0.65-8.48 cm^2 for homogenous and cavitary corpus luteum after subtraction of the cavitary area, respectively; $P>0.05$). The mean volume of all corpora lutea was 5.27 cm^3 (4.64 cm^3 [range 0.67-13.44 cm^3] and 6.12 cm^3 [range 0.68-23.87 cm^3] for homogenous and cavitary corpus luteum after subtraction of the cavitary volume, respectively; $P>0.05$). Statistical analysis showed that females with a corpus luteum with a cross-sectional area ≥2.75 mm^2 were 1.5 times more likely to become pregnant ($P<0.05$) than those with smaller corpora lutea. Similarly, females with a corpora lutea of at least 2.85 cm^3 had a 1.5-fold higher pregnancy rate ($P<0.05$) than females with a corpora lutea of lesser volume.

Conclusions

The analysis of the size parameters of the corpus luteum based on the ultrasound examination performed on the day of embryo transfer may facilitate the selection of recipients with the highest chances of conception. The new, complete assessment criterion, which includes an area and volume of luteal tissue, should be used by veterinarians as part of routine management. Considering that the same method of synchronization was used in all examined recipients and the presence of a clearly expressed estrus was found, the analysis of the size of the corpus luteum should be the decisive criterion for the appropriate selection of recipients and a guarantee of the success of embryo transfer.

1638 - Comparative Evaluation of a Novel Semi-Automated Flock Swab Method and Traditional Cytobrush Technique for Subclinical Endometritis Diagnosis in Dairy Cows—preliminary study

Author: Milena Krupa , Wojciech Barański, Zuzanna Polak, Osvaldo Bogado Pascottini, Geert Opsomer, Dawid Tobolski

Objectives

Subclinical endometritis (SE) in dairy cows poses a considerable challenge in the fields of veterinary medicine and animal husbandry, notably impacting reproduction and animal welfare. The development of advanced diagnostic techniques for the timely and precise detection of SE is essential for improving reproductive strategies and overall herd health. This study introduces and validates *in vivo* a novel semi-automatic method for collecting endometrial cytological samples from dairy cows. This method aims to improve the diagnosis of SE, comparing the cytobrush method, which is currently the gold standard. The study undertakes a comparative analysis of both methods, Method C (cytobrush) and Method F (semi-automatic flock swab), focusing on their accuracy and repeatability in detecting polymorphonuclear (PMN) cells in endometrial smears at thresholds of 1%, 3%, 5%, and 10%.

Material and methods

The study involved 53 Holstein-Frisian cows between 21 and 35 days postpartum, located at a farm in the Warmia and Mazury region. Some cows were checked several times 7 days apart. Endometrial samples were collected from each cow using both the cytobrush and semi-automatic flock methods. The order of collection was randomized to guarantee unbiased first-time usage for each method. The cytological smears were obtained using the cytobrush method as proposed by Kasimanickam (Kasimanickam et al., 2004; Kasimanickam et al., 2005). The innovative semi-automatic method featured a brush with a flexible rod and a swab surface made of soft nylon fibers. An automated rotation system, designed using 3D printing technology, was integrated to facilitate gentle and atraumatic sample collection. A total of 264 cytological smears (Method C n = 132, F n=132) were assessed post Diff-Quick staining, with 300 cells per smear examined under 400x microscopic magnification.

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Results

The study identified 103, 72, 58, and 40 cases exceeding the respective PMN cell thresholds of 1%, 3%, 5%, and 10% using Method C. In contrast, Method F detected 108, 79, 63, and 44 cases ($p<0.05$). We also calculated the sensitivity, specificity, precision,

and F1 scores for Method F, using Method C as a benchmark. Sensitivity varied from 75.00% to 89.32%, specificity from 44.83% to 84.78%, precision from 68.18% to 85.19%, and F1 scores from 71.43% to 87.20%. The relatively lower specificity of Method F is attributed to its higher rate of positive case identification compared to Method C. This aspect is particularly significant considering the lack of visible symptoms in SE and the necessity for a reliable diagnostic technique.

Conclusions

This study demonstrates the efficacy of the semi-automated flock swab method as a viable alternative for diagnosing SE in dairy cows. Its ease of use and safety for animals position Method F as a potential replacement for the traditional cytobrush method, especially for operators with limited experience. The method's accessibility and animal safety make it a valuable tool for SE diagnosis by inexperienced operators or as a substitute for the cytobrush method.

1639 - Do follicle size and coasting time duration significantly influence in vitro embryo production in cattle?

Author: Aleksandra Teresa Pytel -, Piotr Skup, Dawid Tobolski, Krzysztof Papis

Objectives

This investigation was aimed to meticulously scrutinize the impact of ovarian follicle size and the duration of the coasting time (CT) on the efficacy of *in vitro* embryo production (IVP) in cattle. The coasting period refers to the interval following the administration of follicle-stimulating hormone (FSH) without additional hormonal intervention until Ovum Pick-Up (OPU) session. Previous studies by Sirard (*Anim Reprod,* 2019) and Demissie *et al*. (*Trop Anim Health Prod.,* 2022) have described a significant influence of these parameters on the developmental competence of cumulus-oocyte complexes (COCs). Hence, this study was designed to shed light on the developmental potential of oocytes by anlysing variations in coasting time, particularly focusing on periods not exceeding 68 hours as identified by Blondin *et al*. (*Anim Reprod,* 2012) and Nivet *et al.* (*Reproduction,* 2012). Furthermore, we endeavored to clarify the association between follicular size and oocyte competence, as prior research has indicated a positive correlation between larger follicles and enhanced oocyte capability. Ultimately, the goal of this research was to determine the optimal conditions that maximize the production of viable embryos, thus playing a critical role in advancing reproductive technologies in the bovine industry.

Material and methods

We obtained a total of 185 oocytes from Holstein heifers through OPU after FSH treatment, with oocytes of varying quality falling within grades 1 to 4 according to the International Embryo Technology Society (IETS) criteria. The oocytes were subjected to *in vitro* maturation, fertilization, and subsequent culture. The volume of the follicular fluid was classified into three groups based on its quantity, specifically small ($\leqslant$ 0.4 ml), medium (> 0.4, < 0.7 ml) and large ($\geqslant$0.7 ml). The rates of cleavage on day 3 and blastocyst formation on day 7 were documented. To evaluate the impact of coasting time (18, 32, 48 hours), follicle size, and oocyte quality on embryo development, a mixed-effects logistic regression model was employed, with heifer ID being treated as a random variable.

Results

The duration of coasting, which ranged up to 48 hours, and the volume of follicular fluid had no significant impact on the rates of cleavage on day 3 and blastocyst formation on day 7 in the heifers under investigation. However, when the volume of follicular fluid exceeded 1.1 ml a significant decrease in the yield of blastocysts was observed. The success of embryo development on both day 3 and day 7 showed a significant correlation with the grade of the oocyte, with the lowest success rate observed for grade 4 oocytes. Although oocytes of all grades were found in follicles of all sizes, except the follicles with a follicular fluid volume greater than 1.35 ml, which exclusively contained oocytes of the lowest (grade 4) quality.

Conclusions

The results presented here suggest that the greater size of follicles, measured by the volume of follicular fluid (above 1.1 ml), is correlated with a decrease of blastocyst development, underscoring the significance of determining an optimal hormonal

stimulation protocol that would prevent the development of excessively large follicles. The quality of oocytes was confirmed here as a critical factor determining the success of *in vitro* embryo production. Trials with a larger number of oocytes collected after different coasting periods (much longer or shorter) are needed to confirm the influence of CT on blastocyst rate. Further research should consider the detection and/or optimization of multiple factors enhancing the developmental competence of oocytes in order to improve the efficiency of IVP in cattle.

1650 - ASSOCIATION OF PREGNANCY LOSS WITH CULLING IN GRAZING DAIRY COWS

Author: Santiago Corva, German Dominguez, Rodolfo Luzbel de la Sota

Objectives

To assess the risk factors for pregnancy loss and culling in a dairy farm in Buenos Aires, Argentina (~11,000 milking cows).

Material and methods

An observational retrospective study included 25,411 lactation records from 11,175 cows calving from January 1, 2010, to December 31, 2018. Each record had a calving date, lactation number (LACTN), inseminated (AI), pregnant (PREG), pregnancy loss (PREGL), health status (HEALTH; uterine [UTE, retained fetal membranes, puerperal metritis, clinical metritis, clinical endometritis], non-uterine [NUTE, clinical mastitis], both [BOTH], and healthy [HEA]), next calving date (NCD) or date culled from herd (CULL; dead [DDEAD], sold [DSOLD1]; inventory write-off [DSOLD2]). All AI included were after the voluntary waiting period (VWP, 50 d), and pregnancy diagnosis (PD) was performed 28-42 d after AI by ultrasonography. Pregnancy loss was defined as 1) cows that had a dead embryo at PD with ultrasonography 30-60 d after AI, 2) cows with PL after PD detected by visual observation, 3) cows that returned to estrus and were diagnosed not pregnant at the subsequent examination after detected in heat, and 4) cows diagnosed as pregnant and returned to estrus 30 d after PD and were inseminated. The Cox hazard model included only PREG cows, LACTN (1-3) as a stratum to consider repetitions over cows, and PREGL (Y/N) as the response variable. Significance was set at $P \leq 0.05$. Additionally, the average survival time (AST) for CULL (NCD, DDEAD, DSOLD) and the days open (DOPEN) to NCD or CULL were analyzed with a Kaplan-Mayer survival analysis.

Results

The overall CULL rate was 32.80% (8,334/25,441), ranging from 27.80% to 37.27% during the 9 years of the study, and there were significant differences between years ($P<0.0001$). Cows SOLD2 represented 71.66% (5,972/8,334), DEAD represented 22.28% (1,857/8,334), and SOLD1 6.06% (505/8,334). The AST was different for SOLD2 (Median, 95%CI; 219 d, 210-219), for DEAD (29 d, 26-34), and for SOLD1 (409 d, 377-445; $P<0.0001$). Only 60.25% of cows DEAD had a record of disease (1,125/1,857). Cows with clinical mastitis represented 8.3% (94/1,125), metritis 2.9% (94/1,125), lameness 2.2% (25/1,125), sudden death 10.4% (117/1,125), gangrene 7.8 (88/1,125), digestive 8.08% (91/1,125), down cow 7.6% (86/1,125), and others 52.54% (591/1,125). About 30.50% (1,811/5,972) of cows SOLD2 had a disease, 20.83% (1,244/5,972) had lameness, 13.26% (792/5,972) detached udder, 11.68% (698/5,972) infertility, 7.61% (455/5,972) mastitis, and 16.09% (961/5,972) other causes. About 46.12% (3,844/8,334) of CULL cows were not AI, and 53.88% (4,490/8334) had at least one AI, from which only 45.85% (2,059/4,090) were diagnosed PREG. From all cows that were diagnosed pregnant to the first AI that was CULL, 77.02% (1,590/2,059) were SOLD2, 5.49% (113/2,059) were SOLD1, and 17.29% (356/2,059) were DEAD.

The AST to conception (day open) for PREG cows was different for DEAD cows and for cows with BOTH (REP+NREP diseases; 146, 80-251) compared to cows that had a NCD and were HEA (no disease; 79, 77-81; $P<0.0001$). The risk of CULL was 3.29 times higher

(HR) in cows that were DEAD or BOTH compared to cows that had a NCD (HR=3.29, 2.98-3.63; P<0.0001).

Conclusions

Cows with UTE, NUTE, or BOTH health codes had a longer AST than those without them (HEA). Cows with PREGL had a higher risk of being CULL.

1666 - Calving & calf care management workshop – 2. Assessment of neonatal vitality - To decrease prevalence rate of stillbirth

Author: Ottó Szenci Ferenc, David Renaud, John Mee

Objectives

The objective of this workshop is the academic interchange of knowledge on topics pertaining to the periparturient/neonatal period affecting the dam and her offspring with emphasis on knowledge exchange, not lecturing. This abstract, one of three, focusses on assessment of neonatal calf vitality to decrease stillbirth rate in dairy farms.

Material and methods

The workshop format will be that of the World Café methodology. There will be three presenters from Ireland, Hungary, and Canada. The presentations will be preceded by a questionnaire feedback session where the opinions of the participants on the topics of interest will be elicited. The three topics under examination are 1. Prediction of calving, 2. Assessment of neonatal calf vitality and 3. Colostrum management. Each of these topics will be discussed at three workstations, moderated, and recorded by delegates and overseen by the workshop presenters. Following this brainstorming session, presentations will be delivered and the outcomes of the three workstations discussed between presenters and delegates. The workshop will conclude with a summary of the key take home messages and recording of the outputs of the workshop for communication to delegate attendees post-conference.

Results

Stillbirth (perinatal mortality) is the death of a mature fetal calf with longer than 260 days of gestation during calving or within 24 to 48 h of postnatal life. Since a dairy farm's profitability greatly depends on the calves' rate of being born alive and reared to adulthood, therefore if the prevalence of stillbirth rate is higher than $\geq$3% on a given dairy farm, then the farm management has a critical task of decreasing its prevalence rate.

Before starting any obstetrical assistance in anterior or posterior presentation, it is essential to emphasize that we must be 100% accurate in our diagnosis, whether the fetus is still alive or not, to select the most appropriate method for our obstetrical assistance. In doubtful cases, we must use ultrasonography, a pulse oximeter, or measure acid-base balance or lactate concentration to confirm our diagnosis. In cases of severe asphyxia, it is better to perform a Cesarean section to save the fetal life than doing traction, even when the duration would be less than 60 seconds. After calving, several vitality scores may help us evaluate the vigor of a newborn calf. Originally four different clinical signs were recommended to assess the well-being of a newborn calf. Later, five different clinical signs or even more or less were recommended to evaluate the vitality. However, several attempts have been made to create a practical tool to assess newborn calf vitality, a helpful tool that can be used on farms with ease and high accuracy is still missing. Measuring acid-base balance or lactate concentration may increase the accuracy of our diagnosis.

Conclusions

There are already several diagnostic methods to evaluate the clinical signs of vitality during and after calving or to measure acid-base balance or L-lactate concentrations, as well as to use ultrasonography to detect heart rate or pulse oximetry to measure

continuous fetal/neonatal oxygen saturation of arterial hemoglobin and heart rate already on dairy farms. All these methods may contribute to recognizing and eliminating threats to the vitality of the fetal/neonatal calf in time. From this point of view, farm management has the critical task of selecting and applying the methods that can be used most effectively under the given circumstances—considering current economic aspects as well. All these methods can help prevent the damage caused by dystocia, which often contributes to fetal/neonatal mortality. It is essential to emphasize that we must decrease the prevalence rate of dystocia to decrease the occurrence of severe asphyxia. If we cannot avoid the development of asphyxia, we must take care of adequate treatment to reduce its losses. Therefore, this workshop partly focuses on the diagnostic possibilities and limitations of evaluating fetal and neonatal vitality in dairy practices.

1678 - USE OF BUTAFOSFAN AND CYANOCOBALAMIN IN TAI OF HEIFERS

Author: Helio Martins de Aquino Neto, Fernando Andrade Souza, Eduarda Stankiwich Vaz, Natália Santana Siqueira de Lara, Mayara Silvestri, Irma Ximena Barbosa Sanchez, Luciane Maria Laskoski

Objectives

Currently in Brazil, the extensive breeding system is the basis of livestock production, due to low operating costs. However, it is linked to lower profitability, a consequence of the nutritional deficiency of pastures. This occurs due to the seasonal distribution of rainfall and environmental temperatures throughout the year, leading to a reduced supply, in quantity and quality, of forage in certain periods.

Adequate nutrition can stimulate organisms, alleviating negative effects of the environment or, if unbalanced, it can worsen harmful environmental effects, reducing performance and may also have a negative influence on reproduction. Among the nutritional aspects, mineral supplementation constitutes the most relevant input in the composition of the production costs of beef cattle on pastures, highlighting phosphorus, which should be the object of study, to determine its real importance for cattle in terms of gain. weight and reproduction.

The present study aimed to evaluate the effect of supplementation with a product based on butafosphan and cyanocobalamin on the pregnancy rate of ringed heifers submitted to TAI.

Material and methods

Were used 292 24-month-old animals with an average body score of 3.50, being divided into 3 groups: BC - Treatment (78 animals) with the application of 20 ml of product based on 10g of Butafosfam and 0.005g of Cyanocobalamin; CP - Positive Control (79 animals) with application of 20 ml of physiological solution based on 0.9% sodium chloride; and CN - Negative Control (135 animals), without any application. All animals were subjected to the TAI protocol of 4 handlings, with supplementation being applied on D0. After 15 days, they were transferred to bulls until pregnancy was diagnosed, which was performed by rectal ultrasound, 46 days after insemination. The pregnancy rate results were compared using the Fisher's Exact test, with a significance of 5%.

Results

There was no significant difference ($p>0.05$) between the BC (26.92%) and CP (26.58%) treatments. This was observed when comparing both with the negative control (25.92%).

Conclusions

Under the conditions of the present study, the use of supplementation based on Butafosfam and Cyanocobalamin in heifers is not recommended.

1686 - Effects of an injected mineral supplement on thermotolerance and reproductive traits in lactating Holstein cows managed under environmental heat stress

Author: Luis Armando Contreras Méndez, Juan F. Medrano, Milton G. Thomas, Hamad M. Saad, R. Mark Enns, Scott E., Guillermo Luna Nevárez, Pedro Alán López Castro, Pablo Luna Nevárez, , Speidel,

Objectives

A successful reproductive management program during summer in dairy cattle raised in semiarid regions from Mexico is a hard task for producers because milking cows are exposed to an extremely warm environment. An increase in both high ambient temperature and relative humidity induce heat stress (HS) in cattle. Under these conditions, mineral supplementation appears to alleviate the negative effects of HS on cattle fertility. Then, our objective was to evaluate the effects of an injectable mineral supplement (Fosfosan) containing selenium (Se), copper (Cu), potassium (K), magnesium (Mg) and phosphorus (P) on thermotolerance variables and reproductive traits in heat-stressed dairy cows.

Material and methods

Ninety-two freshly calved Holstein cows, with 3 to 7 years old and good body condition score were included in the study. Cows were randomly assigned to a mineral-supplemented (SUP; n=44) or control (CON; n=48) experimental group. Environmental data was collected and used to calculate the temperature-humidity index (THI) that confirmed a severe heat stress during the study (i.e., THI>82 units). As physiological markers of cow's thermotolerance, rectal temperature (RT) and respiratory rate (RR) were assessed biweekly at 06:00 and 16:00 hours. A digital thermometer (TES-1310R) equipped with a contact sounding line (Type K; -com large) that touched the rectal mucosa was used to record RT, while RR was assessed visually by counting the number of flank intercostal movements over a 60-second period (breaths/min). At day 60 after parturition, cows were revised by ultrasound scanning to confirm ovarian activity. Then, cows received a hormonal treatment to synchronize ovulation plus fixed-time artificial insemination (AI) at day 70 postpartum. A blood sample was individually collected 16 days after AI to measure serum progesterone concentrations. Pregnancy diagnosis was carried out by ultrasound 30 d after AI to determine first-service conception rate (FSCR). One-way ANOVA was performed to compare thermotolerance traits and serum progesterone between treatments, whereas a "Chi-square" test analyzed the treatment effect on the trait FSCR. Additionally, correlation and regression analyses were performed to study associations between physiological variables (RT and RR) and serum progesterone concentration.

Results

Average RT during the course of the study was lower in SUP compared to CON group (38.1 ± 0.07 vs. 38.5 ± 0.09; P<0.05); similarly, RR was lower in SUP versus control group (64.2 ± 0.43 vs. 72.5 ± 0.67; P<0.05). Mineral supplementation also influenced positively the reproductive traits as it improved serum progesterone (2.14 ± 0.03 ng/ml vs. 1.32 ± 0.02 ng/ml; P<0.05) and FSCR (54.5% vs. 22.9%; P<0.05) compared to control cows. Pearson correlations showed a moderate association between serum progesterone with RT (r=0.596; P<0.01) and RR (r=0.381; P<0.05) within the SUP group. However, no relationship was detected in control cows between serum progesterone with RT

(r=0.091; P>0.05) or RR (r=0.057; P>0.05). The linear regression analysis detected the RT as predictor for serum progesterone concentration but only in cows receiving mineral supplementation. In these cows, the regression coefficient (B1) indicated a reduction in 0.203 ng/ml in serum progesterone for every unit increase in RT (R^2=0.355).

Conclusions

In conclusion, HS influenced negatively reproductive traits in lactating Holstein cows. However, the injection of a mineral supplement containing Se, Cu, K, Mg and P during the summer appeared to mitigate the adverse environmental effects on cow's thermoregulatory system. As a consequence, supplemented cows improved their ovarian secretion of progesterone leading to an increase in pregnancy rate at first service. Therefore, mineral supplementation during summer is proposed as good strategy to improve reproductive performance in Holstein cows.

1695 - Body condition score, back fat and reproductive performance relationship in Bos indicus in Mexican tropics

Author: Manuel D. Corro Morales, Victoria Blas, Ivette Rubio, Carlos Galina, Clara Murcia

Objectives

The present study *was* carried out to *evaluate* relationship between body condition, back fat and reproductive performance in Bos indicus cows in the Mexican tropics.

Material and methods

A total of 60 multiparous postpartum Bos indicus cows with an average of 166.5 ± 90.6 postpartum days were used. All cows were synchronized using a protocol based on the use of a device with 1.9 g of natural progesterone (CIDR 1900 Cattle Insert, Zoetis, Mexico) and

At CIDR withdrawal, 400 UI IM of ECG were given (Novormon 5000, Zoetis, Mexico) together with 25mg IM dinoprost tromethamine (Lutalyse, Zoetis, Mexico). All cows were inseminated at estrus detected, by the rule AM/PM.

All cows had measurements of body condition score (BCS) scale 1-9, back fat (BF) by ultrasonography, and body weight *(*BW) at three different times during the breeding period. At the beginning of breeding season; at the time of artificial insemination (AI); and at the time of pregnancy diagnosis.

The assessment of BF was carried out using an ultrasound device (Aloka SSD 500, Tokyo, Japan) with a convex transducer and 3.5 MHz frequency to obtain the ultrasonographic images used to measure BF in centimeters. BF was defined as the subcutaneous layer of fat located between the skin and fascia of the longissimus dorsi muscle and measured between the 2nd and 3rd spinous processes of the lumbar vertebrae. Blood progesterone level was assessed at the beginning of pregnancy. All cows were grouped according to the BCS into 2 groups: cows with less than 4.5 (<4 .5) BCS and cows with BCS greater than 4.5 (>4.5). The differences between BCS, BF, and BW, were determined by analysis of variance (ANOVA) Also a linear correlation was performed to determine the association between BCS, BF, and BW.

Results

At the beginning of breeding season 65 % of cows had progesterone levels above 1ng/ml, indicating presence of a corpus luteum. The percentage of cows pregnant after 90 days of the breeding season was greater ($P<0.05$) in the group for >4.5 than group <4.5, 66.7% and 32.4%, respectively. The live weight was 520.1 ±56.6 kg and 438.7 ±55.1 kg for groups <4.5 and >4.5, respectively ($P<0.05$). At beginning of breeding season, BF was 0.731± 0.196 cm and 0.998±0.189 cm ($P<0.05$) for >4.5 and <4.5 groups, respectively. At this moment, also there was a positive relationship between BF and BCS ($r = 0.672$ $P \leq 0.0001$). Additionally, BCS and LW had a moderate positive relationship. (r=0.46- 0.63) (P<0.001).

Conclusions

In conclusion, Bos indicus cows with higher BCS and BF had a better reproductive performance. A moderate to low positive relationship was found between body condition and back fat.

1719 - Evaluation of clinicians and farm veterinarians' clinical approaches to the calving process in Turkey: A questionnaire study

Author: Pelin Erden , Mine Aydemir, İrem Çakmak, Gülnaz Mecitoğlu, Ahmet Gümen, Abdulkadir Keskin, John Mee

Objectives

In this study was aimed to evaluate the interventions and approaches of clinicians (clinic/hospital owners) and farm veterinarians (full-time employees on the farm) before, during, and after calving.

Material and methods

The questionnaire was sent to a total of 490 veterinarians working in different provinces of Turkey, but 430 veterinarians working in 69 different provinces responded and were included in the study (n=339 clinicians and n=91 farm veterinarians). The data were collected between date and date by online questionnaire (Google Forms®). There were questions; to determine the demographic profile in the first part of the questionnaire (n=10); while in the second part, questions were asked about veterinarians' clinical approaches to the calving period (n= 20). The Chi-Square test and Mann-Whitney U test were used to analyses the correlations between variables and to compare responses from clinicians and farm veterinarians.

Results

In total, 96.3 % of the veterinarians participating in the survey were male and 3.7 % were female and the mean (sd) age of the participants was 37±8.7 years. The average duration of professional experience was 12.7±8.7 years. In answer to the question as to whether one should intervene prophylactically at calving, the farm veterinarians (72.5 %) were more likely not to intervene prophylactically (p = 0.001) compared to clinicians (47.5 %). In answer to the question as to whether or not to wait for spontaneous calving where everything seems to be going normally (when the forelimb and nose were visible at the vulvar lips), 69.4 % of clinicians and 96.6 % of farm veterinarians preferred to wait (p < 0.001). When asked about stress due to the calving case difficulties and environmental conditions (barn conditions, animal owner etc.), more clinicians responded that they experienced stress (57.8 %) compared to (p = 0.049) farm veterinarians (43.3 %). The approach to suspending the calf to remove birth fluids was evaluated. More farm veterinarians (34.4 %) did not use this procedure (p = 0.001) compared to the clinicians (17.1 %). Overall, 19.7 % of veterinarians administered analgesic/anti-inflammatory drugs to the cow after eutocia; 80.3 % did not. More farm veterinarians (87.9 %) than clinicians (80.2 %) did not administer these pain medications (p<0.04). Overall, 38.3 % of vets administered antibiotics to cows after eutocia, 42.1 % of clinicians and 24.2 % of farm veterinarians (p < 0.002). While 86.2 % of veterinarians administered antibiotics to cows after dystocia this rate was higher (p < 0.02) in clinicians (88.4 %) than in farm veterinarians (78.0 %). Overall, 48.2 % of veterinarians did not perform any prophylactic practices against retained fetal membranes (RFM) after eutocia, and this did not differ between clinicians and farm vets. In contrast, overall, 75.8 % of veterinarians performed prophylactic measurements to prevent RFM after dystocia and this did not differ between clinicians and farm vets. Regarding the clinical approach to RFM after calving; 39.2 % of veterinarians preferred manual removal, 36.4 % preferred to leave the membranes if manual removal was unsuccessful, and 24.4 % chose to leave the fetal

membranes without manipulation. Nonetheless, 70.0 % of the veterinarians performed manual removal of the fetal membranes before the third day after calving, and almost all of the veterinarians (99.0 %) administered antibiotics in case of high body temperature (>39.5 °C) after manual removal or in cases where the fetal membrane was left without manipulation.

Conclusions

This study demonstrated significant differences in the approaches of clinicians and farm vets to some aspects of calving cases. Clinicians were more proactive to intervene during calving and were more likely to use a calving jack, analgesia and antibiotics than farm veterinarians. Further studies should seek to explore why these differences exist and what implications they have for calving case management.

1742 - Association between the type of vaginal discharge and postpartum endometrial cytology and its effect on the time of pregnancy in dairy cows

Author: DANIEL EDGARDO SCANDOLO LUCINI, Diego Scandolo, Diego Camisasso, Javier Camisasso, Pablo Lopez, Martin Maciel, Edgardo Ortega, Antonella Picca, Pablo Lopez del Cerro, Manuel Casas, Alejandra Cuatrin

Objectives

Clinical and subclinical endometritis in dairy cows are different manifestations of the inflammatory response of the reproductive tract (Pascottini, et al., 2023). When vaginal discharge is diagnosed by Metricheck, without endometrial cytology or biopsy, the source of the purulent material is unknown and that condition refers to purulent vaginal discharge (Pleticha et al., 2009). Subclinical endometritis would be the result of metabolic and inflammatory dysfunction that alters innate immune function and the ability of endometrial PMNs to undergo apoptosis, necrosis, and at last, achieve resolution of inflammation (Pascottini, et al., 2023). The objective of the study was to determine, during the voluntary waiting period, associations between the type of vaginal discharge and endometrial cytology and its effect on pregnancy in dairy cows.

Material and methods

Vaginal discharge (VD) samples from 206 Holstein cows (91 primiparous, 65 second calvers and 50 with three or more parturitions) belonging to a commercial dairy located in Saturnino María Laspiur, Córdoba, Argentina, was performed from 4/1/22 to 6/11/22. Using a Metricheck® at 38±11 days postpartum, the type of VD was determined and classified into four categories: VD0 = clear or translucent mucus, VD1 = mucus containing white or whitish pus flocs, VD2 = discharge containing ≤ 50% mucopurulent material, VD3 = high, containing > 50% purulent material. Furthermore, in order to provide a quantitative assessment of postpartum uterine inflammation, endometrial cytologies (EC) were performed using a modified stainless steel AI gun with a disposable cervical brush at the upper end which was exposed and rotated at the body of the uterus. Therefore, smears were made, fixed and stained with a commercial stain (Tincion 15, Biopur®, Argentina) to count and differentiate 100 cells per preparation by a single observer, with an optical microscope at 1000x magnification, in order to determine the percentage of neutrophils (PMN) over all cells counted. More than 10% PMN was considered endometritis (ESC) and a lower proportion as Normal. The estrus detection and artificial insemination programme started from 45 days postpartum including weekly gynecological examinations using heat synchronization protocols with prostaglandins. The reproductive performance was evaluated with the proportion of pregnancies confirmed until 200 days postpartum. The association between VD, EC and pregnancy was determined using contingency tables. The risk of pregnancy was analyzed using Kaplan-Meier Survival and the difference between groups was determined using the Long Rank test (InfoStat, 2020).

Results

During the postpartum examination, 44.7% of the cows had VD0, 18.4% VD1, 20.9% VD2 and 16.0% VD3. The postpartum day of sampling was similar ($P>0.05$) between categories. An association ($P<0.0001$) was established between VD and EC. 91.3% of the cows with VD0 had normal EC, while the proportion of healthy cows when the discharge was VD1, was 65.8%. On the other hand, between 60.5% to 78.8% of cows presenting

DV2 or DV3 were diagnosed with ESC at CE. The risk of pregnancy from day 45 to day 200 postpartum varied between cows diagnosed with different VD postpartum (P=0.000171). A significant association (P<0.0001) was established between VD and pregnancy at 200 days (P=0.0070). Pregnancy was 92.3% in cows with DV0, 83.8% in those with DV1, 72.5% in those with DV2 and 71.0% in those with DV3.

Conclusions

It is concluded that there is a close relationship between the type of vaginal discharge around 38 days postpartum and endometrial inflammation, assessed by EC, and the amount of pregnancies on a considerable time period; Cows with VD0 or VD1 would present a similar reproductive performance. On the other hand, those with mucupurulent or purulent vaginal discharge would have a lower proportion of pregnancy at 200 days and will require more time to become pregnant.

1751 - FERTILITY OF DUAL PURPOSE COWS (SIMMENTAL & BRAUNVIEH) TREATED WITH OVSYNCH, G-6-G SYNCH AND CIDR+COSYNCH

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Objectives

There are protocols aimed at programming ovulation in milk-producing cattle, to inseminate them at a predetermined time without detecting heat. However, these protocols have not been widely tested in dual-purpose animals; it would be interesting to do so to generate information on the fertility of these animals, especially in cases where it is desired to increase their genetic value. In the present work, the Ovsynch, G-6-G Synch and CIDR+Cosynch protocols were evaluated on the fertility of dual-purpose cattle for 3 consecutive years. The study include cows of the European breeds (Braunvieh & Simmental n=264), which were kept on pasture and their diet was complemented with products to constitute a ration that covered their nutritional requirements.

Material and methods

Animals were treated with Ovsynch, G-6-G-Synch and CIDR+Cosynch. The treatments were applied as follows: Ovsynch, GnRH day 0, prostaglandin F2a (PG) on day 7, GnRH 48 h after PG and insemination 16 to 18 h after treatment without observation of estrus. G-6-G-Synch, PG day 0, GnRH on day 2, GnRH on day 8, PG on day 15, and GnRH 48 h after PG; insemination 16 to 18 hours after treatment. CIDR+Cosynch 100mg of GnRH and CIDR implant on day 0, PG and removal of CIDR on day 7, and 48 h after insemination and application of GnRH16 h.

Results

73.08% of the cows conceived regardless of the protocols used. There were no statistical-significant differences among treatments, nor in the presence of ovarian structures at the beginning of the treatments. Cows in the second year of the study tended to increase fertility compared to those in the first year, possibly related to a greater number of females with body condition between 7 and 8.

Conclusions

In conclusion, there were no differences on pregnancy rates by implementation of Ovsynch or other modified reproductive protocols (G-G-Synch and CIDR-CoSynch) in dual-purpose breeds (Simmental and Braunvieh). More cows become pregnant at body condition scores within 7-8.

1755 - Effect of tail paint removal and type of vaginal discharge at AI on the conception rate of prostaglandin-synchronized dairy cows

Author: DANIEL EDGARDO SCANDOLO LUCINI, Diego Scandolo, Manuel Casas, Antonella Picca, Edgardo Ortega, Pablo Lopez del Cerro, Alejandra Cuatrin, Pablo Lopez, Martin Maciel, Diego Camisasso, Javier Camisasso

Objectives

The use of TAI protocols in European dairy herds began to be questioned due to the substantial time and hormone costs that arise from the routine use (Crowe et al., 2008). In order to reduce expenses, Prostaglandin F2α (PG) is the drug of choice to induce luteolysis in bovine females in oestrus synchronization protocols (Lauderdale, 1975). However, the insemination efficiency after synchronization with prostaglandins is around 70%, where the remaining 30% is not inseminated. These failures may be associated with corpus luteum not receptive at the time of the treatment, to an incomplete luteolysis or the misinterpretation of heat detection aids such as tail painting, among others. The evaluation of vaginal discharge at the time of AI in cows synchronized with prostaglandins, could help to identify part of this population. The aim of the present study was to evaluate and compare, in a herd using tail painting, the conception rate of cows selected to be inseminated, by the characteristics of the vaginal mucosal discharge at the time of inspection, either with or without paint removal.

Material and methods

The study was carried out over 929 artificial inseminations (AI) performed from 08/17/22 to 09/25/22 on 581 Holstein cows (280 primiparous, 174 second calvers and 127 with three or more calvings) with 109 ± 54 days postpartum, on a commercial dairy located in Saturnino María Laspiur, Córdoba, Argentina. Prostaglandins were injected at a weekly ultrasound gynecological examination on cows with a corpus luteum (CL) greater than 20 mm, at the end their voluntary waiting period, on those that were treated the previous week but were not inseminated and the empty females at the pregnancy diagnosis. All cows were tail painted with three different colors; one from parturition to the end of WVP and the other two according if they were inseminated or not. Paint removal observation and insemination was carried out once a day with conventional semen from five different bulls of proven fertility. Prior to AI, vaginal discharge was evaluated in all unpainted cows by rectal manipulation to rule out cows with purulent vaginal discharge, inseminating only those with clear mucus discharge. After 72 hours of prostaglandin injection, cows without paint removal but with clear mucus, were also inseminated. Ultrasound Pregnancy diagnosis was made after 31 days of non-return to service. Contingency Tables (InFostat, 2020) were used to analyze associations between the fertility of the bulls used likewise the conception rate and the insemination with or without paint removal.

Results

No associations were established between the fertility of the bulls and the type of paint removal ($P>0.05$). The 77.0% of the inseminated cows (715/929) were unpainted, while the remaining 23.0% (214/929) were painted but had clear mucus discharge. A positive association was established between depainting and pregnancy ($P <0.0001$). Overall conception rate was 29.7% (276/929), 35.2% (252/715) in unpainted cows and 11.2% (24/214) in those painted ones.

Conclusions

It is concluded that the low conception rate obtained in dairy cows that, at the time of AI, are painted but present a clear mucus vaginal discharge, negatively affects the overall reproductive performance. Even though, few pregnancies could be achieved, a reason to continue with this method, complementary work should be carried out to improve the conception such as the use of GnRH in those without paint removal.

1756 - Seroprevalence of bovine leukosis, bovine viral diarrhea and Neospora caninum and effect on productive and reproductive performance in bovine dairy farms

Author: Cristian Camilo Rúa-Giraldo, Albeiro López-Herrera, Tatiana Ruiz-Cortés

Objectives

Objective: To determine, in dairy cattle from the department of Antioquia (Colombia), the effect of seropositivity to bovine leukosis (BLV), bovine viral diarrhea (BVD) and Neospora caninum(Nc), and its coinfection on productive and reproductive performance

Material and methods

Materials and methods: 599 bovine blood samples were taken from animals in 53 dairy herds. Seropositive and seronegative animals to the three pathogens were determined using the ELISA test. Productive and reproductive information was obtained from 1363 lactation records. The mean of seven productive characteristics: adjusted milk production (PDNa), percentage of adjusted protein (PPa), kilograms of adjusted protein (KgPROa), percentage of adjusted fat (PGa), kilograms of adjusted fat (KgGRAa), cell score somatic characteristics (SCS), and duration of lactation (DurL), all adjusted to 305 days, as well as the mean of four reproductive traits: number of services per conception (S/C), age at calving (AgeP), days open (DA) and calving interval in days (IEP) were associated with seropositivity to BLV, BVD and Nc and their interaction was analyzed using a generalized linear model (GLM).

Results

Results: The presence of BLV was significant ($p<0.05$) for KgPROa, KgGRAa, where the average was lower in infected cows, and for IEP ($p=0.05$) being significantly higher in infected cows. BLV seropositivity impacted DA ($p=0.093$) and IEP ($p=0.05$). The Nc seropositivity was significantly associated with S/C ($p=0.008$); BVD seropositivity had no significant effect on any reproductive parameter evaluated. When the effect of seropositivity to two pathogens was evaluated, animals seropositive for BLV and BVD were significantly affected in the IEP ($p = 0.05$). Seropositivity to the three pathogens (BLV, Nc and BVD) was not significantly associated with S/C ($p = 0.198$), AgeP ($p = 0.349$), but there is a tendency to affect DA ($p = 0.069$) and IEP ($p = 0.061$).

Conclusions

Conclusion: BVD seropositivity affected the levels of KgPROa, KgGRAa; a trend was observed towards a negative effect of the interaction between the three pathogens evaluated on the DA and IEP of dairy herds, as well as the interaction between BLV and BVD on the IEP, which can negatively affect the profitability of dairy herds. These results show the importance of implementing a program to control and prevent the spread of these pathogens to avoid the associated economic losses.

1758 - Dominance in goat bucks affects sexual behaviour, but not semen quality during an estrus synchronization protocol in the breeding season

Author: Carlos Luna-Palomera, Fernando Sánchez Dávila , Keyla Mauleón-Tolentino, Estela Garza Brenner, Javier Hernandez-Melendez, Cecilia C. Zapata-Campos

Objectives
The aim of the present study was to determine the effect of dominance in adult male goats on sexual behavior, semen quality and pregnancy percentage in an estrus synchronization program in goats during the reproductive season.
Material and methods
Six adult bucks with a live weight of 33.15±8.5 kg and a body condition of 2.75±0.4 were used; they were divided into 3 groups (n=2) by introducing a dominant buck (DB) and a subordinate buck (SB), grouped with 10 adult goats/group. Females were synchronized with a 5-day protocol using CIDR vaginal devices, with 3.75 mg cloprostenol and 300 IU eCG (equine chorionic gonadotropin) administered at the time of the device removal. Both females and bucks were unilaterally identified so that the sexual behaviour of each buck within each group could be observed through 8 security cameras for a period of 36 hours. Similarly, a semen sample was collected using an electroejaculator to determine semen quality for a period of 36 hours at 6-hour intervals. In addition, pregnancy was determined by real-time ultrasound 30 days after estrus syncrhonization.
Results
Partial results indicate that the percentage of pregnancy was not different ($P>0.05$) among the groups of goats studied, with a result of 70% for group I and 80% for groups II and III. There was a higher number of matings/ejaculate (MEs) for group I (21) vs. 17 MEs for group III and 11 MEs for group II ($P<0.07$). Similarly, the number of MEs for DB was 74 vs. 15 MEs for SB ($P<0.001$). On the other hand, regarding the sexual behavior, there were differences in dominance effects for lateral approaches, mating attempts, mating, mating/ejaculate, which were higher for the DB ($P<0.001$) compared to the SB. Semen quality was not affected both within group and for dominance effect ($P>0.05$). However, over time, semen quality was affected for both mass and progressive motility ($P<0.05$).
Conclusions
It is concluded that the dominance effect affects sexual behavior but not semen quality; however, it remains to be determined how it would affect the offspring when paternity tests are performed once the offspring of this experiment are born, as well as to determine testosterone and cortisol concentrations.

1768 - Comparison of two commercial sample-based pregnancy tests in dairy farms

Author: RAFAEL PAIVA

Objectives

The objective of this study is to compare the performance of 2 commercial kits for pregnancy detection using the inhouse quantitative ELISA as the gold standard.

Material and methods

Samples were collected weekly from a dairy operation, with 6,000 milking cows. They perform weekly blood-based pregnancy tests in the cows, between day 28 and 35 after Artificial Insemination, processing the samples in their on-site ELISA laboratory using a commercially kit [BioPRYN®] (Kit No. 1), another commercially kit [Alertys™ IDEXX Ruminant Pregnancy Test] (Kit No. 2) was run in parallel. A total of 2,292 samples, were run with both kits.

Blood samples were collected by the dairy personnel thru the caudal vein every Tuesday morning using Vacutainer® tubes with no anticoagulant (red cap tubes) and Vacutainer® needles.

The in-house quantitative ELISA was performed in Pohler's lab (Texas A&M University, Animal Science) to determine the concentration of PAGs using a monoclonal-based PAG ELISA using a polyclonal antibody (Ab 63) to quantify PAGs secreted early in gestation, with a sensitivity of 0.28 ng/mL.

Results

In the ROC curve, Kit 2 has a better performance at any PAG level, this means that there will be better results and less error in Kit 2.

Sensitivity and Specificity for Kit 1 is 88.89% and a Sp of 94.59%, respectively, Kit 2 has a Se of 89.23% and a Sp of 95.89%, Positive and Negative Predicted Value (PPV, NPV) for Kit 1 is 97.70%, and 76.75%, for Kit 2, 98.26%, and 77.36% respectively.

The GLIMMIX model performed when only discrepancies were used, and using the In-House as a Gold Standard, showed statistical differences (P-Value < 0.01) on the samples with false positive or false negative diagnosis were Kit 1 have 46.4% and Kit 2 have 16.7%, and in the samples with Re-Check diagnosis with 72.7% and 21.5% for Kit 2 and Kit 1 respectively. It is important to understand that a Re-check diagnosis is something desirable, when the cow has a PAG level where the test cannot clearly determine if its pregnant it should classify as re-check, in this way other tests must be performed 7-10 days later, and no hormones, especially if $PGF_{2\alpha}$ is applied in a possible pregnant cow. The concentration of PAG's at day 31 of gestation is significantly higher in cows that maintained the gestation (9.58 ± 0.31; mean ± SEM; $P < 0.05$) when compared with those that lost the gestation between day 31 and 59 (4.15 ± 0.33; mean ± SEM) {K.G.Pohler, 2016 #23}. The correct interpretation for a recheck result is that an embryo death has a high probability to occur, and another test must be run in 7 days to confirm the diagnose. The results obtained comparing Kit 2 and Kit 1 observed for True Positive (66.7 vs 36.3%), True Negative (93.3 vs 84.0%), False Positive (33.3 vs 63.4%), False Negative 6.7 vs 16.0%), Open with Re-check result (70.3 vs 68.4%), and Pregnant with Re-check (29.7 vs 31.6%).

Conclusions

Kit 2 has 0.34 % higher Sensitivity and 1.3 % higher specificity when compared to kit 1, this means that more accurate results are obtained with kit No 2 vs kit No 1.
Kit 2 performs with higher Se, Sp, PPV and NPV than Kit 1.
Kit 2 has better performance at low PAG levels (beginning of gestation) and at high PAG levels (late gestation), accuracy in early gestation is very important for decision making and management of the open cow.
Using only the discrepant samples between both tests, P-Values of < 0.01 were found on samples with false positive or false negative results (16.7% for Kit 2 vs 46.4% for Kit 1), and in the recheck samples (72.7% for Lit 2 vs 21.5% for Kit 1). Recheck results are important to identify cows where embryo is dying, producers can create an alert list with cows that need to be tested one week later to measure embryo mortality and make decisions in open cows that will reduce open days.

1779 - Effect of GnRH injection at AI on the conception rate of tail painted with clear mucus vaginal discharge vs tail unpainted dairy cows

Author: DANIEL EDGARDO SCANDOLO LUCINI, Diego Scandolo, Alejandra Cuatrin, Pablo Lopez del Cerro, Manuel Casas, Antonella Picca, Edgardo Ortega, Diego Camisasso, Javier Camisasso, Pablo Lopez, Martin Maciel

Objectives

Cows with a higher heat expression intensity have higher conception rates than those with a lower one (Burnett et al., 2022). A higher demonstration is also associated with a greater number and percentage of collected and viable embryos and with a better ovulatory response (Madureira, et al., 2020). The use of GnRH or its synthetic analogues, increases conception in cows with lower heat manifestation, but does not show benefits in those with higher expression (Burnett et al., 2022). The aim of the present study was to evaluate and compare the conception rate of cows, submitted to a “tail paint” heat detection programme, selected to be inseminated by a clear mucus vaginal discharge even if they appeared unpainted (heat) or not which were injected with a GnRH analogue at the time of AI.

Material and methods

The study was carried out over 969 artificial inseminations (AI) performed from 9/26/22 to 11/27/22 on 353 Holstein cows (181 primiparous, 101 second calvers and 71 with three or more parturitions) with 94 ± 36 days postpartum (range 46 - 198 days) belonging to a commercial dairy located in Saturnino María Laspiur, Córdoba, Argentina. Prostaglandins were injected at a weekly ultrasound gynecological examination on cows with a corpus luteum (CL) greater than 20 mm, at the end their voluntary waiting period, on those that were treated the previous week but were not inseminated and the empty females at the pregnancy diagnosis. All cows were tail painted with three different colors; one from parturition to the end of WVP and the other two according if they were inseminated or not. Paint removal observation and insemination was carried out once a day with conventional semen from five different bulls of proven fertility. Prior to AI, vaginal discharge was evaluated in all unpainted cows by rectal manipulation to rule out cows with purulent vaginal discharge, inseminating only those with clear mucus discharge. After 72 hours of prostaglandin injection, cows without paint removal but with clear mucus, were also inseminated and received a single injection of 10.5 µg of Buserelin Acetate IM in order to induce ovulation. Ultrasound Pregnancy diagnosis was made after 31 days of non-return to service. Contingency Tables (InFostat, 2020) were used to analyze associations between the fertility of the bulls used likewise the conception rate and the insemination with or without paint removal

Results

The 87.7% of the inseminations (850/969) were performed in “tail unpainted” cows, while the remaining 12.3% (119/969) in “tail painted” ones but presenting a clear mucus vaginal discharge at the time of the inspection. A positive association was established between depainting and pregnancy (P <0.0001). The overall conception rate was 38.9% (377/969), 41.1% (349/850) and 23.5% (28/119) in “tail unpainted” and “tail painted” cows respectively.

Conclusions

It is concluded that the conception rate of “tail unpainted” cows with mucous vaginal discharge at the time of insemination was higher in relation to “tail painted” cows that received a GnRH analogue injection. However, the application of GnRH improves de conception rate in such a practice where cows with clear mucus vaginal discharge but “tail painted” are also inseminated. This suggests that its administration in cows with a lower intensity heat expression, could be successfully adopted to increase reproductive performance of the herd.

1784 - A gene bank of cattle skin cells as caryoplasts for Interspecies Somatic Cell Nuclear Transfer (bovine/ovine) and the ability of sheep oocytes to reprogram bovine somatic cells nuclei

Author: MARÍA DEL CARMEN NAVARRO MALDONADO, IVÁN AGUILAR CHÁVEZ, DEMETRIO ALONSO AMBRÍZ GARCÍA, SALVADOR ROMO GARCÍA, NELSON MANZANARES MIRANDA, JOSÉ ROBERTO VAZQUEZ AVENDAÑO

Objectives

The objectives of this study were, first, to create a gene bank with skin cells of cattle of different age, sex, breed, productive purpose, alive or *Postmortem* and, second, to evaluate the ability of sheep oocytes to reprogram a World Champion Simbrah cow´s somatic cells, in an interspecies Somatic Cell Nuclear Transfer (iSCNT).

Material and methods

Primary somatic cell cultures were generated from ear skin samples from a Simmental bull, a Simbrah cow and a Tuli heifer, owned by the Universidad Autónoma de Nuevo León located near de city of Linares, Nuevo León, México. Also, a Holstein calf and an Angus cow owned by the Facultad de Estudios Superiores Cuautitlán - UNAM located in the state of México, México were sampled. All experiments for cell culture and iSCNT were performed at the Assisted Animal Reproduction Laboratory of the Universidad Autónoma Metropolitana Iztapalapa Unit (UAM-I) located in México City. All cattle were selected on basis of their conformation and outstanding productive characteristics. For all specimens, 0.5cm^2 ear skin samples were taken using a “punch” applied in the back of one of each animal´s ear and transported for 2-13 h, to UAM-I in tubes containing DMEM supplemented with 10% fetal bovine serum (DMEMs). The samples were processed by 2 different methods: enzymatic disintegration with collagenase type I and II (0.02%) incubating them for 2 h at 38.5°C in an oscillatory chamber inside a stove. Skin cells were obtained in 3 mL of recovered culture medium, and by mechanical disintegration (manual fragmentation of the sample into smaller portions using a scalpel at room temperature). Skin fragments were incubated in DMEMs at 38.5°C. 5% CO_2 at saturation humidity for 7 days or until somatic cells were derived in a primary culture. Then cells were subjected to 3 passages. Samples from each passage were cryopreserved in liquid nitrogen tanks at -196°C as gene bank, until use. Subsequently, oocytes were aspirated from sheep ovaries, obtained from a local slaughterhouse. From the oocytes obtained, those surrounded by a minimum of 4 layers of cumulus cells were subjected to *in vitro* maturation (IVM) incubating them for 24 h at the previously mentioned conditions in TCM-199 supplemented with hormones and EGF. Matured oocytes were subjected to cumulus cells retrieval using hyaluronidase (to confirm the MII phase by the presence of the first polar body) and to zona pellucida retrieval using pronase before manual enucleation. Groups of two enucleated oocytes (using the Handmade Cloning technique or HMC), were used as cytoplasts and a somatic cell obtained from enzymatic disintegration of the World Champion Simbrah cow was used as a karyoplast. The two cytoplasts and nuclear donor cell (triplets) were fused by a single pulse of 100 V/mm DC for 9 µseg, the reconstructed embryos were chemically activated and cultured in Cleavage media until they developed to the morula stage.

Results

Enzymatic and mechanical methods allowed a population from 1,504,077±849,313 to 2,578,281±851,437 ear skin cells (considering the 3 passages) whether the samples were from young animals, adults, females, male, of any breed, live or *Postmortem*. The skin cells derived morphology was corresponding to fibroblasts. The time needed to achieve results with the enzymatic method was 11.5± 3.9 days, and 12.4±3.4 days for the mechanical method ($p>0.05$, Student's t test). About IVM, out of 169 sheep ovaries were collected, obtaining 232 oocytes. From these, 90 matured oocytes (that reached the MII or Metaphase II phase shown by the expansion of cumulus cells surrounding the oocytes) were retrieved, representing 39% of the total sample. After fusion of triplets, 97% Simbrah cloned zygotes were reconstructed by iSCNT. Simultaneously, ovine parthenogenetic embryos were generated as controls. The results obtained for cleavage [55% cloned (33/60) vs. 65% parthenogenetic (36/55)] and early embryo development showed a higher numerical difference for iSCNT, compared to parthenogenic embryos that reacheaad the morula stage [46% (15/33), vs. 16% (6/36), respectively, $p<0.05$, Student's t test], but not at the blastocyst stage [0% (0/33), vs. 54% (19/36), respectively, $p<0.05$, Student's t test].

Conclusions

We conclude that it was feasible to create a gene bank with cattle skin cells and it was possible to demonstrate the ability of sheep oocytes to reprogram these, producing Simbrah cloned morulae through HMC and iSCNT.

1799 - A short superovulation protocol using FSH and ECG in Romosinuano cows in a tropical environment.

Author: María Yanory López Gómez, Salvador Romo, Antonio Parlange, Froylan Sosa, Michael Kjelland

Objectives

The Romosinuano is a *Bos taurus* breed genetically adapted to tropical conditions, but little has been done to preserve this genetic material as embryos. It is known that two of the sources of variation in Embryo Transfer programs (ET) are the breed and the type and dose of Follicle-Stimulating Hormone (FSH) to induce superovulation. The objective of this study was to evaluate the response of Romosinuano cows to a short protocol of superovulation and fixed-time embryo transfer using a combination of FSH/Luteinizing Hotmone (LH) [Stimufol™] and Equine Chorionic Gonadotropin (eCG) [GonActive™].

Material and methods

Five Romosinuano donors with an average age of 4.5-5 years, 45 days post-calving, with body condition score of 2.5-3 (scale 1-5) were used in this study, in the Mexican State of Veracruz. This study was carried out during the months of April and May, a period of drought in the tropical region of the Gulf of Mexico, with temperatures as high as 40 °C. Prior to the start of the study, cows were evaluated by ultrasound to determine the presence of a corpus luteum and/or follicles larger than 10 mm in order to rule out pregnancy and anestrus. The treatment was assigned to the donors, consisting in total FSH doses of 300 mg (3.3 ml) administered in a 2-day, twice a day protocol, as follows: Day 0: Intramuscular injection of 3 mg of Estradiol Benzoate [EstroActive™] and insertion of a CIDR with 1.9 g of Progesterone [Zoetis™]. Day 4: Mornig: FSH, Afternoon: FSH. Day 5: Morning: FSH, Afternoon: FSH. Day 6: Morning: Two doses at once of a Synthetic Prostaglandin F_2alpha [InducelActive™] and eCG (400 IU). Day 7: CIDR is withdrawn. Day 8: Morning: Buserelin Acetate 0.01 mg [LiberActive™], Afternoon: Fixed-Time Artificial Insemination (FTAI), first Artificial Insemination (AI) was 24 hours after CIDR withdrawal or 12 hours after Buserelin. Day 9: Morning: FTAI, second AI 12 hours later. Day 15: embryo collection and evaluation. For AI, frozen conventional semen in 0.5 ml straws (30 million sperm per straw) from two Romosinuano bulls was used. Semen was evaluated with a field microscope, showing a minimum post-thaw motility of 40%. The AI and embryo collection procedures were carried outby a single technician.

Results

The results of the first program, performed in April, had an embryo production rate of 30 viable embryos with 6 embryos/donor on average compared to the procedure in May, with results of 18 viable embryos with 3.6 embryos per donor on average, respectively. The two programs performed produced a total of 48 viable embryos collected in 2 attempts, using the same 5 donors, with an average of 4.8 viable embryos/donor. The program performed in April had a higher embryo production rate compared to the one in May. There were differences in the two programs evaluated, and overall, the transferable embryo production was acceptable compared with results obtained in other breeds, but similar when compared to responses obtained with tropically adapted *Bos*

taurus breeds. Based on these results, the protocol evaluated can be used with donors of the same age range, expecting similar results, with the advantage of the lower cost and easier management of this short protocol. The results also seem to indicate that season is another cause of variation in superovulatory treatments and embryo production, even in breeds genetically adapted to tropical environments.

Conclusions

In conclusion, the FSH/LH [Stimufol™] hormones used in this research, showed good response to superovulation and total viable embryos produced, comparable to other hormonal products. Further study is required using statistical analysis for evaluating differences in environmental factors.

1810 - CURRENT STATUS OF CATTLE EMBRYO PRODUCTION IN MEXICO.

Author: Salvador Romo García, Salvador Romo Domínguez

Objectives

The production of bovine embryos is essential for genetic improvement and to increase the production of high-quality animal foods that contribute to satisfy the growing national demand for animal protein. In Mexico there are different teaching and research laboratories, commercial laboratories, companies and/or professionals producing embryos *in vivo* and *in vitro*, and performing embryo transfer. The objectives of knowing this information are to facilitate communication between institutions and professionals, to carry out collaborations in academic or research projects. By knowing the names and locations of the different laboratories and professionals in this activity, it is possible to initiate contacts to learn about the opportunities that each one offers for working with producers, with companies and with the local and national livestock industry.

Material and methods

Mexico has a network of contacts made up of colleagues who work in Embryo Technologies, this is the Mexican Embryo Technology Association (META), affiliated with: the International Embryo Technology Society (IETS), and collaborating with the Embryo Production and Transfer Statistics, and with the Partner Societies Committees, to prepare world statistics and different partnership matters. In the most recent report from the IETS (2022), Mexico ranks fifth in the world in the number of bovine embryos produced and transferred, with a total of 73,997, behind the fourth place that is Colombia, with 82,339, the third place that is Argentina with 105,766, the second place Brazil with 464,417, and the first place which is the United States, with 901,815 (Viana JHM, 202**3**).

Results

Nationwide, there are 14 teaching and research laboratories for the *in vitro* production of bovine embryos in Mexico, most of them belonging to national or state funded universities, or to official institutions. There are also 14 privately owned commercial laboratories for the *in vitro* production of bovine embryos, distributed all over the country. Also, there are 58 different private companies and professionals that produce bovine embryos *in vivo* and that also transfer embryos produced *in vivo* or *in vitro*. These companies and professionals will be listed by names and by regions of the country according to their geographical location: Northeast (14), North (4), Northwest (8), Center (4), West (4), Central Gulf (8), South and Southeast (16), for a total of 58. According to the IETS 2022 statistics report (Viana JHM. 2023), Mexico ranks fifth in the total number of embryos produced worldwide (including *in vivo* produced and *in vitro* derived), with a total of 73,997. This is a reduction in contrast with the 2021 report, in which Mexico ranked fourth, with 97,671 (Viana JHM. 2022). These numbers of embryos were produced by a total of 86 units distributed all over the country, divided in teaching, research and commercial laboratories (28) and companies and professionals in the country (58). However, it is important to mention that not all of the previously mentioned institutions and professionals report their embryo production numbers.

Conclusions

It can be concluded that the information provided in this presentation helps facilitate communication between institutions and professionals, to carry out collaborations in

projects in private practice, academia or research. The knowledge of the names and locations of the different professionals and laboratories in this activity makes it possible to start contacts in order to learn about the opportunities offered to work with producers, universities, companies and with the livestock industry at local, regional and national levels.

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1816 - THE IMPACT OF TIMING AND ADMINISTRATION OF EQUINE CHORIONIC GONADOTROPIN ON PREGNANCY RATES IN BRAFORD CATTLE UNDER FIXED-TIME ARTIFICIAL INSEMINATION PROTOCOL IN THE MEXICAN TROPICS

Author: CARLOS HERNANDEZ LOPEZ, RENE CARLOS CALDERON ROBLES, RENE CALDERON CHAGOYA, ADRIAN GUZMAN SANCHEZ

Objectives
This study aimed to evaluate the effect of equine chorionic gonadotropin (eCG) either 48 hours before or on the day of intravaginal progestin device removal on ovarian follicle diameter and pregnancy rate per service in a fixed-time artificial insemination (FTAI) protocol in Braford females (cows and heifers).
Material and methods
The study was conducted at the commercial farm "San Rafael" in Hueytamalco, Puebla, Mexico, characterized by a humid subtropical climate. Thirty-six Braford females were selected and categorized based on their physiological status (cows and heifers) and body condition score (BCS: 0 to 9 points), and assigned to three homogeneous groups. The cows and heifers were examined by transrectal palpation and ultrasonographic examination (Aloka 500 ultrasound with 7.5 MHz transducer) to measure the structures in their ovaries. Group 1 (eCG6) received equine chorionic gonadotropin (eCG) 48 hours before CIDR removal, Group 2 (eCG8) received eCG at CIDR removal, and Group 3 (control) did not receive eCG but had a corpus luteum. All animals were given a progestin intravaginal device (CIDR) containing progesterone (1 mg), followed by benzoate estradiol (2 mg) on Day 0 of the synchronization protocol. On Day 6, the eCG6 group (N=12) and eCG8 group (N=13) received 300-400 IU of eCG for heifers and cows, respectively, whereas the control group (N=11) did not receive any eCG treatment. CIDR withdrawal occurred on Day 8 followed by a dose of PGF2a containing 150 mcg of D-Cloprostenol. On Day 10, all animals received buserelin acetate (16.8 mcg), and fixed-time artificial insemination (FTAI) was performed by a single technician using semen from proven bulls. Pregnancy detection was conducted 41 days after the first insemination by transrectal palpation and ultrasonographic examination. Pregnancy rates were analyzed with the GENMOD procedure of Statistical Analysis System software (SAS), a binomial distribution was specified and a logit link function was used in the analysis of pregnancy rate (logistic regression analysis).
Results
The pregnancy rate for the eCG6 group was 33%, for the eCG8 group was 46%, and for the control group was 18%. Results showed that administering a dose of eCG at CIDR removal produced significantly higher pregnancy rates compared to the control group. However, no significant differences were observed between the eCG6 group and the other groups. Additionally, there were no significant differences in pregnancy rates between cows and heifers. The presence of the calf was found to have a significant effect on the pregnancy rate. The size of the follicles was not significant for the model.

Conclusions
In summary, the study concluded that there were no significant differences in pregnancy rates between the eCG6 group and the other groups, nor were there significant variations between cows and heifers. However, the presence of the calf significantly affected the pregnancy rate, while follicle size did not show significance in the model.

1824 - Reproductive performance of dual-purpose cows using synchronization and artificial insemination protocols in the tropics of Mexico

Author: Ivette Rubio, Edith Robledo, Manuel D. Corro

Objectives

Reproduction efficiency in dual-purpose cattle raised under tropical conditions is low. Due to the presence of prolonged postpartum anestrus, caused, among other reasons, by deficiencies in nutrition and by the prolonged effect of calf suckling. One of the ways to solve this problem is the promotion of reproductive management with the strategic use of synchronization protocols and artificial insemination. The present study was to evaluate the reproductive performance, using synchronization and artificial insemination protocols in dual purpose cattle raised under tropical conditions production units in the north-central region of the state of Veracruz.

Material and methods

Eleven small dual-purpose cattle production units (SDPU) were selected out of a l of 45 SDPU in south east Mexico. From a total of 115 cows, only 60 cows were selected based on body condition and reproductive status. Three reproductive programs were applied: the first used a progestin-based synchronization protocol with fixed-time insemination (FTAI). The second was an OVSYNCH with GnRH-PG with FTAI; and artificial insemination group implemented at estrus detection without heat synchronization.

Results

The gestation rate obtained was 58%, 64% and 44% for progestogens, GnRH-PG and natural estrus, respectively. A general average of gestation was 55 % (P>0.05)

Conclusions

The use of synchronization programs and artificial insemination in cattle in tropical areas is feasible despite existing limitations. And they are an appropriate tool to improve reproductive performance in UPBDPs in the tropics.

GOATS

1141 - Clinical and diagnostic study of Caprine Udder Contagious pustular dermatitis

Author: Karima Akool Al Salihi, Entissar Mansour Abdul Rasool

Objectives

Objectives: This study intends to report the clinical and diagnostic investigation of contagious pustular dermatitis in the udder of goats, which was reported as an outbreak in the herds of goats in Al Muthanna province/ Iraq in the winter of 2022. In addition, it investigates the histopathological changes of the udder biopsy collected from an affected goat.

Material and methods

Outbreaks description: Twenty-two goats from two flocks were included in the study. The lesions were located on the udder/teat skin (22/22). Udder skin lesions were irregular in size and shape. Lesions appeared circular, nodular, and ulcerate, enclosed with a crust, and associated with swelling supramammary lymph node. Some lesions showed large necrotizing ulcers with undetermined edges associated with regional lymph node enlargement. The goats suffered from fever and congested mucus membranes. The goats also suffered from tender udder accompanied by pain and refused to suck off their kids, suffering from starvation. They were then followed by retention of milk and mastitis. Samples were collected from the affected areas, kept in 10% neutral buffered formalin, transferred to the histopathological laboratory, processed with routine histopathological procedures, stained with H&E, and examined under light microscopy.

Results

Results: Examination the sections of the udder's skin reveals various histopathological features. The sections showed prominent areas of orthokeratotic and parakeratotic hyperkeratosis. Moreover, the epidermal hyperplasia with prominent rete ridges extending into the dermis layer was also seen. The stratum spinosum showed degenerative changes, with numerous distended, vacuolated cells having pyknotic nuclei. Limited keratinocytes confined large, eosinophilic intracytoplasmic inclusion bodies. Additionally, pustule formation was observed at the margins of the lesions. The underlying dermis displayed broad subacute inflammation, with moderate to marked aggregations of a combination of inflammatory cells and tissue necrosis.

Conclusions

In conclusion, this study reported outbreaks of contagious pustular dermatitis on the goat's udder. The diagnosis was based on clinical examination and histological changes. Detection of this disease is vital for effective, successful disease control to reduce farmers' economic losses, maintain animal health, and avoid significant zoonotic disease implications.

1229 - Serologic surveillance for Caseous Lymphadenitis and Johne's disease on a Korean native goat farm

Author: Chan-Lan Kim, Kwan-Woo Kim, Eun-Do Lee, Sang Uk Chung, Bong-Hwan Choi, Yeoung-Gyu Ko, Changyong Choe

Objectives

Caseous lymphadenitis(CLA) is a bacterial infectious disease by *Corynebacterium pseudotuberculossi(C. pstb)* and commonly observed in goat farms, showing over 50% herd level seroprevalence in South Korea. This disease presents a high morbidity and economic losses including reduced meat and milk production and increased labor losses for treatment. Also Johne's disease(JD), *Mycobacterium avium subsp. paratuberculosis*(MAP) infection contributes to decreased productivity of goat production. In Korea, CLA or JD vaccines are not approved and then only disinfection is important management for prevention. Therefore the surveillance of the two diseases is absolutely required for the control and prevention. The objective of this study is to evaluate whether it is appropriated to use commercial ELISA kits against CLA and JD for serological monitoring without cross-reactivity.

Material and methods

In a closed goat herd, serum antibody titer against *C. pstb* or MAP was evaluated at three times six months apart using ELITEST CLA kit(HYPHEN BioMed, FRANCE) or Mycobacterium paratuberculosis Antibody Test kit(IDEXX Laboratories, USA) respectively. The experimental procedure and interpretation of results were performed according to the manufacturer's instructions, except that positive value against *C. pstb* was determined by double point of cut-off value calculated according to the manufacturer's instructions because of high background absorbance.

Results

At the first serological monitoring, JD and CLA seroprevallence was 3.3%(5/150) and 43.3%(65/150) respectively. All sera of individuals(12 head) having subcutaeneous abscesses by palpation showed CLA-positive results, and any CLA sero-negative goats did not have such a abscess. After 6 months later, JD and CLA seroprevallence increased to 3.95%(7/177) and 50.8%(90/177) respectively. Thereafter the subsequent culling of JD-positive goats was conducted, and one time whole-herd antibiotic injection every month was administered for three times until next examination. As a result, at the third surveillance JD-positive ratio decreased to 1.26%(2/159) and CLA-positive ratio decreased to 37.1%(59/159). Interestingly among total 486 sample, JD-positive sera represented CLA-positive results by 50%, and CLA-positive sera showed 3.3% JD-positive results. These results suggest that JD usually make co-infection with *C. pstb.*

Conclusions

Once subcutaneous abscesses is present in a herd, there might be about 5 times sero-positive goats such as this goat farm. JD is not a notifiable disease until several years old. In conclusion, after JD ELISA test, CLA serologic survey with JD-positive sera would be recommended for cost-saving JD and CLA eradication programmes, if CLA seroprevallence is high such as the goat farm tested in this study. And the regular antibiotics treatment would be worth considering to prevent the spread of CLA, in a high contaminated herd with *C. pstb*.

1274 - Control Coxiella burnetii in three Q fever-infected goat herds through the implementation of a vaccination program

Author: Raquel Toledo Perona Toledo Perona, Jesús Gomis Almendro, Juan José Quereda Torres, Marion Toquet, Nerea Bailon Larrañaga, Antonio Contreras de Vera, Philippe Gisbert, Ángel Gómez Martín

Objectives

Q fever is a worldwide zoonotic disease produced by the bacteria *Coxiella burnetii* (*Cb*). Coxiellosis has an important public health relevance due to the diverse number of transmission routes, high spread capacity, and wide range of hosts. Goats are considered the main source of transmission of the disease for humans. There are few studies on the impact of vaccination on excretion in goats and there is a need to evaluate the results of vaccination when the entire herd is vaccinated following a clinical outbreak. Furthermore, the simultaneous presence of *Cb* in the nasal cavity and lungs of infected goats has recently been reported (Toledo et al., 2023). Despite of, the relevance of these findings is unknown. For that reason, the first study's objective was to evaluate the influence of Q fever vaccination on immunity, as well as on the dynamics of excretion of the bacteria through different excretion routes in goat herds that vaccinate their entire population after a clinical outbreak. The second objective was to study in these herds the presence of *Cb* in environmental samples as bedding and troughs as well as in domestic animals in contact with the infected animals.

Material and methods

Three goat herds with high abortion rates (50-70%) were included in the study. Animals were vaccinated with an inactivated *Cb* phase I vaccine, (Coxevac®, Ceva Santé Animale). According to the manufacturer's instructions, 2 subcutaneous injections 3 weeks apart were given In one herd, a high number of weak born kids were also observed before vaccination. Between 11 and 16 animals (n=41) were sampled in each herd from the beginning of the study (T0). Sincesome animals died throughout the study, the number of animals sampled can differ over time. Blood samples were obtained for serology as well as milk, vagina, feces and nasal samples for qPCR. The same animals as well as environmental contamination were monitored over time at T0 (during the first week after the abortion, before the primary vaccination), T1 (two months after revaccination) and T2 (300 days after the primary vaccination).

Results

All animals in T1 seroconverted, maintaining over 70% in T2. After two months of second doses of vaccination (T1), there was a 70% reduction in shedding patterns over time. The presence of animals in which *Cb* was identified was anecdotal (only one goat) 300 days after the three herds implemented the vaccination (T2) program for the entire group (with symptoms and without symptoms; adults and young).. Similar results were obtained in the study of Arricau-Bouvery et al., 2005 in goats, where authors reported a vaginal and fecal shedding reduction after vaccination. In our study, nasal samples had the highest frequency of *Cb* detection in T0 and T1, followed by fecal, vaginal, and finally milk samples. Regarding environmental contamination, all the herds presented positive samples at T0 (bedding, troughs, dogs and chickens), and even in T2, two farms still showed positive samples (troughs).

Conclusions

This study described a *Cb* shedding reduction and high seroconversion rate in vaccinated goat herds after a clinical Q fever outbreak. Moreover, these results highlight the importance of vaccination programs in order to control Q fever infection and contamination in goat herds. This study has shown that there is an underestimated relevance of nasal excretion in goats. Nasal samples could improve the sensitivity of Q fever diagnosis.

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1336 - Isolation and frequency of caprine herpesvirus type 1 in Mexico.

Author: JAZMIN ARMENDARIZ DE LA LUZ, JOSE FRANCISCO RIVERA - BENITEZ, ITZEL ESCOBEDO-ALVAREZ , ANDRES ERNESTO DUCOING-WATTY

Objectives

The objective of this work was to isolate caprine herpesvirus type 1 and determine the frequency in different goat production units.

Material and methods

Obtaining samples. 500 goats from Mexico City, Guanajuato, Sinaloa, Puebla, Zacatecas, Aguascalientes, Querétaro, Hidalgo, Morelos, Jalisco, Nayarit and the State of Mexico were selected, from whom a vaginal swab sample was taken using sterile swabs of dacron immersed in cellular maintenance medium and frozen immediately in liquid nitrogen until use.

Polymerase chain reaction (PCR). From the previously described samples, DNA extraction was carried out using the QIAGEN commercial package and a fragment of 1125 base pairs of the gene that codes for glycoprotein C was amplified. For pfPCR, the Promega Green commercial package was used. master mix with the following quantities Master Mix 12.5µl, forward primers 2 µl, reverse primer 2 µl and sample DNA 4.5 µl. The thermocycler constants used were the following: initial denaturation temperature 95º for 15 minutes, 35 cycles of denaturation temperature 95º for 60 seconds, alignment temperature 56º for 60 seconds and extension temperature of 72º for 60 seconds, finally, extension final of 72º for 5 minutes. The pfPCR products were evidenced by horizontal electrophoresis in 1.5% agarose gel, stained with ethidium bromide and observed in a digital photodocumenter.

Viral isolation. Vaginal swab samples positive for the presence of HvCa-1 were selected to perform viral isolation in monostearates of the caprine choroid plexus (SCP) cell line. The samples were filtered and confronted to the cell monolayer with serum-free medium for 30 minutes, then medium with 2.5% fetal bovine serum was added and kept in an incubator with a CO2 atmosphere and at 37ºC for 96 hours. The bottles were observed directly under the microscope every 12 hours until the characteristic cytopathic effect of the virus was observed, which is cell lysis and formation of lytic plaques.

Results

Of the 500 goats sampled, it was determined that 67% (335/500) are subclinical infected with caprine herpesvirus type 1. Frequencies of 1.1 to 50% have been reported throughout the world, where they have described that the highest percentage of goats are subclinically infected and in the case of the most associated clinical signs are respiratory and reproductive ones. The present study showed that 100% of the production units studied have goats infected with the virus in a high percentage of between 42.5 and 88.8% in the States of Guanajuato and Querétaro, respectively. Hao et al in 2020 in China found that only 46% of the production units included in their study had goats infected with the virus. These data demonstrate the wide spread of the virus in our country. These same authors conclude that the presence of more pronounced clinical signs is favored by coinfection with other viral agents and Pollock, et al in 2019 determined that HvCa-1 infection in goats is observed subclinically and that when faced with stress problems clinical signs will begin to appear. Both data are of utmost

importance, since both factors that trigger the accentuation of clinical signs are present in goat production units in Mexico. With respect to viral isolation, they have described that the main cell lines for isolation are bovine kidney cells (MDBK). In this study we performed the isolation in ovine choroid plexus cells (PCO) and from 12 hours post infection we observed the cytopathic effect of cell rounding, lysis and formation of lytic plaques.

Conclusions

The present study confirm the natural circulation of caprine herpesvirus type 1 in goat production units in Mexico City, Guanajuato, Sinaloa, Puebla, Zacatecas, Aguascalientes, Querétaro, Hidalgo, Morelos, Jalisco, Nayarit and the State of Mexico. The virus infection in goats is identified subclinical, however its excretion through the vagina is confirmed, which indicates that if problems of this type occur in the production unit they could be associated with their infection, likewise. , we rule out its presence in other organs or systems such as the respiratory tract. In addition to this, it is important to highlight that factors such as stress and the presence of other infectious agents can trigger more pronounced clinical signs, which is why it is important to work on welfare and biosecurity measures within goat production units in Mexico.

1338 - Clinical study and molecular identification of poxviruses in goat milk production in Mexico.

Author: JAZMIN ARMENDARIZ DE LA LUZ, VIRIDIANA COLLAZO-DOMÍNGUEZ, EDUARDO MARTIN CABRERA-DOMÍNGUEZ, JOSE FRANCISCO RIVERA-BENÍTEZ, ANDRES ERNESTO DUCOING-WATTY

Objectives

Identify the presence of poxvirus in muco-cutaneous lesions in goats from Mexico.

Material and methods

Obtaining samples Swab sampling of mucocutaneous lesions and scabs from 301 goats that were in intensive production units for milk production in the states of Mexico City, State of Mexico, Querétaro, Zacatecas, Guanajuato, Aguascalientes was carried out. , Sinaloa and Hidalgo. Once the swab was taken, it was immersed in cell maintenance medium and preserved in liquid nitrogen until processing.

Description of clinical cases. The production units considered in this study had a previous history of papular lesions and crusts in the mucocutaneous junctions of the oral cavity. During sampling, papular lesions and crusts were recorded in the oral cavity, nose, coronary region of thoracic limbs and nipples.

Polymerase chain reaction (PCR). From the previously described samples, DNA extraction was carried out using the QIAGEN commercial package and a fragment of 600 base pairs was amplified. For pfPCR, the Promega Green master mix commercial package was used with the following quantities: Master Mix 12.5 µl, forward primers 2 µl, reverse primer 2 µl and sample DNA 4.5 µl. The thermocycler constants used were the following: initial denaturation temperature 95º for 15 minutes, 35 cycles of denaturation temperature 95º for 60 seconds, alignment temperature 52º for 60 seconds and extension temperature of 72º for 60 seconds, finally, extension final of 72º for 5 minutes. The pfPCR products were evidenced by horizontal electrophoresis in 1.5% agarose gel, stained with ethidium bromide and observed in a digital photodocumenter.

Results

95% (285/301) of the goats sampled were positive for the presence of poxvirus, confirming that the lesions identified were caused by this virus. All production units were positive for the presence of the virus with frequencies from 87.5% to 100%, with the state of Hidalgo being the one that showed the lowest frequency and the States of Zacatecas and Aguascalientes those that had 100% of infected animals. with the virus. In Mexico in 1987, Tótora, et al reported the presence of specific antibodies against the virus in goats in intensive production units dedicated to dairy production. Based on both results, it is confirmed that poxviruses have been circulating for around 40 years. natural in Mexican goat production causing negative effects on milk production and being a great risk factor for human health due to its zoonotic potential. In other Latin American countries, such as Brazil, frequencies of 42% have been reported, mainly in lactating goats. In comparison with our results, we obtained high frequencies in adult goats.

Conclusions

The natural infection of poxvirus is confirmed in dairy goats in which crusty lesions were mainly identified in the oral cavity, teats and coronary labrum, determined as characteristic lesions of the virus. These results show the need to implement preventive

medicine and biosafety programs in intensive production units that allow us to control this viral agent in Mexico to avoid the negative impact at a productive, economic and health level.

1451 - Frequency of Small Ruminant Lentivirus (LvPR) in goat and sheep production units in Mexico.

Author: Itzel Guadalupe Escobedo Álvarez, ANDRÉS ERNESTO DUCOING WATTY, Jazmín De la Luz Armendáriz, José Francisco Rivera Benítez

Objectives

The aim of this study was to identify the serological and molecular presence of LvPR and determine the frequency of its presentation in sheep, goat and mixed production units in the central region of Mexico.

Material and methods

Sampling was carried out aimed at the presence of clinical signs associated with LvPR infection. Two hundred and eighty-four samples were obtained from goats and 194 samples from sheep. The samples obtained were whole blood by jugular venous puncture to obtain serum and peripheral blood mononuclear cells. For serological diagnosis, the competitive ELISA test was performed, using the commercial package Ruminant Lentivirus, Antibody Test Kit, cELISA (VMRD Inc. USA) (Ghanema et al 2009; Ramírez et al 2011); The development and use of the Kit was carried out in accordance with the manufacturer's instructions. DNA extraction was carried out from the peripheral blood mononuclear cells (PBMC) samples obtained using the commercial package DNeasy blood & tissue kit (QIAGEN). With this DNA obtained, the endpoint polymerase chain reaction test was performed to amplify a fragment of the long terminal region of 233 base pairs using the primers previously described by Sánches, et al. To carry out the test, the Kit Go taq Green commercial package from promega was used. The reaction mixture for the PCR were: Master Mix 6.5 µl, Fw 1µl, Rv 1µl and ultrapure H2O 1.5 µl. The constants for amplification: first cycle of denaturation 94° C for 5 minutes, denaturation 94°C for 30 seconds, alignment 60 °C for 40 seconds, extension of 72 °C for 30 seconds (these three for 35 cycles) and finally a final extension cycle of 72 °C for 5 minutes. Data obtained were evaluated through descriptive and Ji square analysis using JMP vewrsion 17.2 (SAS Inst. Inc., 2023).

Results

Fifty four percent of goats and sheep (211 and 48 respectively) presented specific antibodies against gp135 of LvPR. Eighty six percent of goats and sheep (257 and 155, respectively) were positive for the presence of the viral genome. The signs most frequently observed were the respiratory (139 goats and 139 sheep) in the three types of production: goats, sheep and mixed. The second sign with a number of animals was arthritis (114 animals) found only in goat systems as well as mastitis (86 animals), which agrees with most of the reports published about LvPR in goats. Encephalitis (10 animals) was the minor sign observed and only found in sheep.

Conclusions

The frequencies found in these herds were high for both species, which indicate that there is a presence of LvPR in goat and sheep flocks in the central region of Mexico These results show that it is important to implement control, prevention or eradication strategies, considering the limitations and resources of each type of production and system, with the objective of avoiding further spread of the disease, through the use of serological techniques (ELISA) and molecular (PCR) for diagnosis. As a follow-up to these

frequency studies, it would be considered to carry out genotyping of the samples obtained, in order to know the main genotypes present and which animals they are mainly affected, in terms of ages or species, in order to confirm that interspecies transmission is of medical importance.

1506 - Associations between serum gamma-globulin concentration, enzyme activities, growth and survival in preweaning Alpine goat kids

Author: Marilena Bolcato , Mariana Roccaro, Maria Giulia Ferrari, Francesco Dondi, Arcangelo Gentile, Angelo Peli

Objectives
Colostral immunity plays a crucial role in the well-being of young ruminants, yet there has been limited exploration into the specific factors influencing passive transfer status and its impact on the early growth of goats. In different ruminant species, colostrum enzymes that cross the intestinal barrier have shown potential as indicators of passive transfer status and their determination may be a viable alternative to the expensive measurement of immunoglobulin G (IgG). This research seeks to analyse the influence of variables such as sex, litter size, dam parity, and birth weight on passive transfer status in Alpine goat kids. The study also aims to analyse correlations between gamma-globulin concentration (GG) and growth performance before weaning. Additionally, the investigation delves into the potential of serum total protein concentration and colostrum enzyme activity (including γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)) as predictive markers for passive transfer status in neonatal goat kids.
Material and methods
Sixty-six Alpine goat kids (39 males, 27 females), born to 28 does at one dairy goat farm during two delivery seasons, were included in the study. Kids nursed their dams in group housing until weaned at 50 days of age. Blood samples were collected 24 h after birth. Body weights (BW) were taken at birth and weaning. Serum enzyme activities and total protein concentration were measured using a clinical biochemical analyser. Serum GG was determined by gel electrophoresis. Statistical analysis was performed using GraphPad Prism (v. 8.2.1).
Results
No significant differences in serum GG between males and females, singlets and twins, multiparous' and primiparous' kids were found. No association was detected between birth BW and GG. Serum GG was strongly and significantly associated with TP (R2 =0.85; p 0.0001) and moderately associated with GGT (R2 =0.47; p 0.0001). No correlation was found with ALP, AST, and LDH. Although partial failure of passive transfer (FPT) was diagnosed in 23% of kids, no effects on morbidity (3%), mortality (0%) and pre-weaning growth performance were observed.
Conclusions
Our findings support the use of serum total proteins as an indirect means of estimating immunoglobulin concentration. Contrarily, the effectiveness of predicting passive transfer status is limited when relying on the measurement of serum gamma-glutamyltransferase (GGT). The use of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) as indicators for passive transfer status is not recommended. Ultimately, failure of passive transfer (FTP) is not

necessarily related with the health and preweaning growth performance of Alpine goat kids raised in non-intensive breeding systems that adhere to good farming practices.
Please note that the contents of this abstract have already been published:
Roccaro M., Bolcato M., Ferrari M.G., Dondi F., Gentile A., Peli A. «SMALL RUMINANT RESEARCH», 2023, 227, Article number: 107064, pp. 1 – 7.

1509 - Mastectomy in a pet goat after severe necrotic mastitis with postoperative generalised emphysema

Author: Rok Marzel , Jože Starič, Lena Veren Geč, Primož Klinc

Objectives

Precocious udder is one of the seemingly harmless conditions that can occur in non-pregnant female goats. It is a condition in which either one or both mammary glands may produce milk or a milk-like fluid. Normally the milk is reabsorbed if the udder is left alone, but mastitis can also occur. Medical treatment is usually unsuccessful, and mastectomy is often the treatment of choice.

Material and methods

A radical mastectomy was performed on a 10-year-old doe (Boer x Saanen) with chronic suppurative mastitis due to a precocious udder with a fistulous abscess. The animal was sedated with xylazine (0.1 mg/kg intramuscularly), and general anaesthesia was achieved by intravenous administration of ketamine (2 mg/kg) via a jugular catheter. Ketamine was administered at the same dose every 15–20 minutes throughout the procedure to maintain the surgical depth of anaesthesia. After the surgical site was shaved and disinfected, an elliptical skin incision was made around the udder. The mammary gland tissue was bluntly excised from the skin together with the abscess. As a lot of skin was removed around the abscess, firm skin tension was required to close the wound. A drain was placed at the surgical site to remove excess fluid and the animal received daily repeated antibiotic and anti-inflammatory treatment.

Results

On the fifth day after surgery, the wound healed well and the drain was removed. A small amount of serous fluid (up to 3 ml) and some gas, both without odour, oozed from the drainage site. Palpation revealed generalised emphysema. Although the body temperature was normal, the antibiotic treatment was extended by 5 days and the doe was kept for observation. Three weeks after the procedure, the wound was completely healed. Mild subcutaneous emphysema was still palpable on most of the back and rump. The doe had an excellent appetite, was bright, alert and responsive and was discharged from the clinic.

Conclusions

Keeping traditional farm animals as pets is becoming increasingly popular. Therefore, the treatment approach must also change, as the life quality of the animal takes precedence over the production value. It is important that these keepers are familiar with the diseases of farm animals, as the udder can develop and thus mastitis can occur, even if the doe is kept in the presence of a castrated male or is the only goat in the household.

Chronic mastitis, gangrenous mastitis that does not respond to medical treatment and injuries, as well as a precocious udder and udder tumours are the most common clinical conditions for which unilateral (partial) or total (radical) mastectomy is indicated. It is a safe and effective treatment that improves quality of life and is particularly appreciated in companion animals.

The unexpected complication after the surgery we performed was subcutaneous emphysema, which was not only around the surgical site but was generalised. We suspect that air had entered through the drainage, especially as the formation of the

subcutaneous emphysema began to decrease the next day after the drainage was removed. As the general condition of the animal improved and the emphysema regressed without impairment, the doe finally left the clinic 3 weeks after admission.

Keywords: mastectomy, generalised emphysema, pet doe

1529 - Evaluation of different population densities and feeder lengths and their effect on behavior in dairy goats under confinement conditions

Author: Mariana Hernández Castañeda, ANDRÉS ERNESTO DUCOING WATTY, Javier Gutiérez Molotla, Rosalba Soto González

Objectives

The goat is a source of meat, milk and fiber around the world, for this reason it lives in different types of production systems. The demand for products of animal origin has increased in recent years due to population growth. Specifically, products derived from goats have generated interest because they can be consumed by people intolerant to bovine dairy products. Given the current demand for goat dairy products, production systems have implemented zootechnical practices to have milk all year round. These managements can be: having very large groups of goats in confined systems, changes in feeding, reproductive management, regrouping at sensitive times. This can prevent the goats from expressing the behavior of the species and the social structure becomes unstable due to resource competition. In Mexico there is very little information on the recommendations for available space per goat and also on the length of the feeder under confinement conditions.The aim of this study is to evaluate the effect of different individual spaces and feeder lengths per animal on the behavior of goats under confinement conditions.

Material and methods

The study was developed at the Center for Practical Teaching and Research in Animal Production and Health from the College of Veterinary Medicine, UNAM. Fifty-two French Alpine, Saanen and Toggenburg adult goats in production and in the lactation rest period were evaluated in two different periods, considering two different individual spaces (6 and 3.5 m^2) and two feeder lengths (40 and 60 cm) per animal. Preliminary observations and two moments of observation were carried out. Each treatment had two repetitions. Changes in agonistic, individual behavior and some affiliative behaviors were measured. Continuous sampling was carried out for the events and scan sampling for the different states observed. Data obtained in the present study was evaluated using a model for repeated observations using the JMP 17.2 statistical package (SAS Institute Inc. 2023).

Results

The results indicated that for aggressive behavior, the length of the feeder did not have a significant effect ($P>0.05$) on the behaviors lunging, heading, pushing, and threatening, while the frequency of the behaviors heading ($P=0.0183$), threatening ($P=0.0156$) and grooming ($P=0.0153$) were significantly greater when the space per goat was smaller (3.5 m^2). It could also be observed that the length of the feeder and the space per goat did not have a significant effect ($P>0.05$) on the proportion of individual behaviors (eating, resting, standing, ruminating and drinking).

Conclusions

The effect of individual space per goat did not make a difference for most aggressive behaviors. However, a significant difference was found when the space per animal was 3.5. m^2 for heading and pushing behavior, compared to 6 m^2 per animal. Feeder length did not have an effect neither on the frequency of aggressive nor on affiliative behaviors. In practical terms, the feeder length and population density treatments did not have a

significant difference in individual, aggressive and affiliative behavior. We could say that under the particular conditions of the production system of the present study, the space available per animal and the length of the feeder did not show significant effects on behavior, which could suggest that both conditions provide the environment for the goats to have a good level of animal well-being, since they express behaviors typical of the species.

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1536 - Homeopathic intervention in dairy goats infected by Small Ruminant Lentiviruses improves clinical and ultrasound parameters.

Author: Lorena Jamila Alves Ferreira Guimarães, Letícia Dos Santos Steves, Tatiane Pacheco Barenco de Oliveira, Raymundo Rizaldo Pinheiro, Maria Leonora Veras De Mello, Juliana Patrão de Paiva, Carla Holandino, Argemiro Sanavria, Nathalie Costa da Cunha, Mário Felipe Alvarez Balaro

Objectives

Small ruminant lentiviruses (SRLV) are chronic and progressive diseases that compromise the production, health and animal welfare. Due to the lack of cure, vaccines or any effective treatments against lentiviruses, homeopathy emerges as an efficacious and safe intervention. The promising results of homeopathic products in veterinary medicine have motivated the present clinical trial which was developed following a triple-blind, placebo-controlled protocol. This study sought to evaluate the effect of two homeopathic interventions to treat goats naturally infected with SRLV: bio therapy (living nosode) and a homeopathic complex.

Material and methods

This study was approved by an ethical committee for animal use (number: 1988141022). A total of 42 adult Saanen goats (4.6 ± 1.3 years old; 2.6 ± 0.2 body condition score [BCS]; 59.5 ± 8.7 kg) with chronic clinical signs of arthritis and weight loss were included in this study after confirmation of infection (PCR) and/or seropositivity (Western Blot) to SRLV. The three following experimental groups were organized: SRLV living nosode (G_{nosode}; n=14), Placebo ($G_{placebo}$; n=14), and Homeopathic ($G_{homeopathic}$; n=14). The homeopathic medicines were prepared following the Brazilian Homeopathic Pharmacopeia, and the living nosode was compounding using a standard sample of Caprine Lentivirus (CAEV-Cork; B1 genotype) as active substance. The complex was prepared by equal parts of the following homeopathic medicines: *Rhus toxicodendron* 12CH, *Ledum palustre* 5CH, Calcaria carbonica 12CH, *Bryonia alba* 5CH and *Pulsatila nigricans* 30CH. The placebo animal group received the solvent of homeopathic medicines (ethanol 5%) following the same posology. Treatments were administered daily, orally, for 90 days. Every month, all goats underwent a clinical examination, collection of production indices and joint ultrasound. For comparison among groups, analysis of variance (ANOVA) was used followed by Tukey's test at a significance level of 95%.

Results

There was a treatment effect ($p<0.05$) for productive (BCS and live weight) and clinical indices (carpal joint index, crepitation and width indices). G_{nosode} and $G_{homeopathic}$ showed a greater BCS when compared to $G_{placebo}$ (2.7±0.3 = 2.8±0.4 ≠ 2.5±0.3). $G_{homeopathic}$ had a greater live weight compared to $G_{placebo}$, even though equal to G_{nosode} (62.4±11.2 ≠ 57.2±9.5 = 60.4±9.8 kg). In clinical evaluations, G_{nosode} obtained lower carpal joint indices when compared to $G_{placebo}$, even though equal to $G_{homeopathic}$ (8.2±1.7 ≠ 8.7±1.0 = 8.5±1.4 cm). There was also a statistical significant improvement ($p<0.05$) for joint ultrasound indices (sagittal and transverse section of the carpus, distance from the joint capsule to the subchondral space, area and lesion perimeter). Such indices obtained lower values in one or both treated groups (G_{nosode} and $G_{homeopathic}$) when compared to $G_{placebo}$. The values obtained among groups for the distance from the joint capsule to the subchondral space (6.9±3.3 = 7.6±3.4 ≠ 10.8±4.4 mm), area (137.5±84.4 = 157.9±89.1 ≠

235.0±90.1 mm) and joint lesion perimeter (81.8±18.8 = 85.7±17.6 ≠ 101.5±15.9 mm) are highlighted. Comparing the joint lesion area from D0 to D90, there was a reduction in lesions by more than 50% in $G_{homeopathic}$ (↓50.1%) and G_{nosode} (↓51.3%) with an increase in $G_{placebo}$ (↑37.2%). Considering the lesion perimeter, there was also a reduction in $G_{homeopathic}$ (↓25.9%) and G_{nosode} (↓26.3%) with an increase in $G_{placebo}$ (↑3.4%). At the distance of the capsule to the subchondral region, there was a reduction in $G_{homeopathic}$ (↓34.4%) and G_{nosode} (↓41.7%) with an increase in $G_{placebo}$ (↑43.2%).

Conclusions

G_{nosode} and $G_{homeopathic}$ demonstrated promising outcomes both improvement of arthritic lesions and in the production rates. Therefore, both medicines, under the current conditions, can be safely used in goats infected with LVPR displaying chronic arthritis and weight loss.

1545 - Characterization of the small ruminant lentivirus capsid protein (SRLV-rp25) coupled to immunostimulant complexes based on glycyrrhizinic acid

Author: María Azucena Castañeda-Montes , Julieta Sandra Cuevas-Romero, José Luis Cerriteño-Sánchez, Hugo Ramírez-Álvarez, Rocío Lara-Romero

Objectives

The objective of the study was to evaluate the antigenicity of the recombinant protein p25 of Small ruminant lentivirus (SRLV) and its incorporation into immunostimulant complexes formulated by glycyrrhizinic acid (GA) liposomes and evaluation of the humoral and cellular response in mice immunized with the immunostimulant complexes.

Material and methods

In previous works, the ORF of the p25 protein was amplified from the viral strain FESC-752, cloned into the expression plasmid ChampionpET-SUMO and a strain producing the p25 protein was obtained (BL21 of *E. coli*). Subsequently, p25 protein was expressed using IPTG as an inducer and p25 protein production was analyzed by SDS-PAGE and Western-blot. The protein was purified using Ni-NTA affinity chromatography. Analysis of epitope distribution was performed using DNAstar's Protean program. For the analysis of the antigenicity of the p25 protein, 98 plasma samples from goats and sheep from various states of the country, naturally infected with SRLV, were analyzed. The preparation of GA liposomes (GAL) was carried out using the lipid firm hydration method, working with the ratio (2:1:2) of saponin: cholesterol: phospholipids using GA (Sigma-Aldrich) as saponin. The immunogenic characteristics were determined by immunizing 5 groups of 10 F1 male mice, 21 to 28 days old, each group, which were inoculated subcutaneously using the following doses of 5 µg/animal of: p25 protein with GA, GA alone (negative control), p25 protein with PBS, p25 protein with ISCOM adjuvant, p25 protein with GAL and PBS alone. Blood sample was obtained from the mice through the tail vein to recover serum and determine IgG production. The kinetics of antibody production were measured by indirect ELISA and plotted for evaluation of seroconversion.

Results

Using Western-Blot, it was determined that 500 ng is the ideal concentration to carry out antigenicity studies. The p25 protein has regions with high antigenic potential that are exposed on the surface. The ability of the recombinant protein to recognize infected animals was analyzed using plasma samples from goats and sheep naturally infected with SRLV and it was observed that 61% (n = 60) of the plasma samples from infected goats and sheep were able to recognize to infected animals. recombinant p25 protein to different degrees. The protein was used to immunize mice, the antibodies obtained recognized the p25 protein during the days of immunization and increased dramatically over time. In the three groups of mice immunized with the protein-adjuvant mixture, the production of anti-p25 antibodies was greater than in animals immunized with the protein alone, suggesting that the protein-adjuvant mixture enhances the immune response to generate antibodies. However, the protein itself is capable of generating an immune response in animals. This confirms that the recombinant protein produced has the necessary elements to generate a response in mice, even without adjuvant,

compared to negative control mice. Serum from control animals inoculated with GA did not show significant increases in optical density at O.D. throughout the experiment and these levels are considered as test background. To determine the cellular immune response, the levels of three different cytokines were evaluated: IL-4, IL-10 and IFN-γ. The results showed similar levels of IL-10 in mice immunized with the SRLV-rp25 protein and with GA ($P>0.05$). As well as similar lower levels of this interleukin in the groups immunized with the GAL-SRLV-rp25 and ISCOM-M®-SRLV-rp25 complexes. The difference in the levels of IL-10 produced by SRLV-rp25 alone compared to the levels of the complexes formed by GAL-SRLV-rp25 was highly significant ($P<0.001$). On the other hand, none of the groups showed a difference in the production of IFNγ, this result suggests that these cells are not playing an important role in the cellular immune response stimulated by the administered immunostimulant complexes. Likewise, serum IL-4 values were measured although the presence of this cytokine was not observed.

Conclusions

The recombinant p25 protein bound to the complex of AG nanoparticles, ISCOM and liposomes from *Quillaja saponaria* are highly immunogenic compared to the use of the protein alone, indicative of obtaining satisfactory results, for future evaluations. in small ruminants.

1636 - A cases report of congenital goiter in Boer goat kids in Poland

Author: Grzegorz Balicki, Bartłomiej M. Jaśkowski, Jakub Kulus, Marek Gehrke, Jędrzej M. Jaśkowski

Objectives

Goiter is one of the manifestations of iodine deficiency. In Poland, its occurence is unlikely due to constant iodine supplementation and – in many areas – the relatively short distance from the sea. Another reason may be the consumption of goitrogenic substances. Racial predispositions to goiter have been demonstrated in many goat breeds. They seem likely due to the relatively small population of goats of some breeds. Cases of goiter in Boer goat kids are presented below.

Material and methods

Case 1

On May 18, 2021, the five-year-old goat A, a multiparous Boer breed, gave birth to three kids. Two males and one female. All of them had a severely enlarged thyroid gland. The first male was born dead, the second died after 4 hours, and the female survived for 21 hours. The father of the kids was a five-year-old Anglo-Nubian goat. The offspring of other goats in this farm mated with this ram did not show any disease lesions. Post-mortem and pathological examinations were performed on the kids including thyroid measurements. Hyperplasia of thyroid follicular epithelial cells and a reduced number of follicles containing colloid were confirmed.

Once again, goat A gave birth in March 2023. This time three female kids were born. Initially, two goats were born easily, one of which was alive and the other dead. The third one was born dead after many hours of labor. The living kid did not differ in appearance and development level from her peers not affected by this condition.

Case 2

In May 2022, on the same farm, a sister of goat A gave birth. The litter consisted of three male kids. The first kid died after 2 hours, the next one after an hour, and the third one, resembling an anasarca, was born dead. As in the previous case, all individuals had clear goiters.

Results

Blood was collected from two goats – goat A, and a control goat whose offspring were born at the same time and which did not show goiter, and from two kids, a goat with goiter from mother A and a control goat B without mentioned changes. In goats A and B thyroid-stimulating hormone (TSH) and thyroxine (T4) levels were 0.606 ng/ml and 6.35 µg/dl, and 0.681 ng/ml and T4 were 6.78 µg/dl, respectively. In the blood of a kid with goiter (A) and without goiter (B) the TSH and T4 levels were 0.105 ng/ml and 9.41 µg/dl, and 0.087 ng/ml and 6.48 µg/dl, respectively. The iodine concentration in the blood of a goat kid (A and B) was 86.25 ug/l and was slightly reduced.

Conclusions

The mothers of the goat kids were Boer goats. Their father was an unrelated male goat of the Anglo-Nubian breed. The interview data show that in kids born to goats mated with the mentioned goat, cases of goiter have never occurred in the offspring. In the described cases, a strongly enlarged thyroid gland suggested a simple iodine deficiency as the cause of goiter. An important clinical manifestation of iodine deficiency in goats is a reduced level of synthesis of triiodothyronine (T3) and T4 hormones, which causes

an increase in the synthesis and production of TSH. The inability to produce thyroid hormones causes hypertrophy of thyroid cells and compensatory enlargement of the gland. In this case, goiter is more likely to be caused by abnormal thyroglobulin biosynthesis. Some facts may indicate such a genesis: a) relatedness of the mothers, b) use of the same goat for mating, and c) quite significant relatedness of Boer goats in Poland. The gene encoding thyroglobulin is responsible for the function of the thyroid gland. Disturbances in its structure may cause hormonal dysfunctions – a lack of T3 and T4 synthesis, which results in goiter. On the other hand, in the discussed case, a genetic background of goiter in the offspring of Boer goats cannot be ruled out.

1687 - Plasma testosterone of well-fed male goats decreases due to prolonged exposure to solar radiation

Author: LUZ MARIA TEJADA UGARTE

Objectives

Objective: In the subtropical north of Mexico most goats are managed in the semi-extensive production management system. Under this system, females and males graze between 5 to 8 kilometers daily eating only available natural vegetation without supplementary feed. There are factors such as the nutritional status of the males, or exposure to high environmental temperatures that could modify the reproductive efficiency of the male. Undernourished male goats exhibit reduced testicular size and plasma testosterone concentration. The Comarca Lagunera (26ºN) is characterized by a dry and semi-arid climate. In this dry region the temperature is warm almost all year. The prolonged hours under direct solar radiation exposure by these animals could reduce testosterone concentrations in males. The purpose of the current study was to determine whether plasma testosterone of well-fed goats decreases due to prolonged exposure to solar radiation compared to well-fed male goats provided with shade.

Material and methods

Materials and methods: A total of 10 local adult goats from the Comarca Lagunera, 3 years old, were used. The males were distributed in two groups (n = 5 each) homogeneous regarding body weight, testicular diameter. The control group was housed in a 6 x 6 meter pen provided with shade, while the experimental group was housed in a 6 x 6 meter pen without providing shade, so they were exposed directly to sunlight throughout the study in order to simulate the same hours of exposed solar radiation as the male goats managed in the semiextensive production management system. All males were fed with 2 kg of lucerne hay/animal.day (9.6 MJ/kg, 18% crude protein/kg dry matter) which provided a maintenance diet, mineral salts in blocks and clean water ad libitum. The two groups of males were exposed to natural changes in environmental temperature throughout the study.In both groups, plasma testosterone concentrations were determined every two weeks from 11 months and were determined in blood samples obtained by jugular venipuncture in tubes containing 30 µg of heparin. The blood samples were centrifuged at 2500 X g for 20 min, and the obtained plasma was stored at -20 ^{o}C until assayed by RIA in a single assay.

Results

Results: Plasma testosterone concentrations varied over time ($P < 0.0001$), and there was a significant interaction between time and the groups bucks ($P < 0.001$). In control bucks, testosterone concentrations were higher than experimental bucks in June, September, December and January ($P < 0.05$).

Conclusions

Conclusion: In summary, plasma testosterone of well-fed bucks decreases due to prolonged exposure to solar radiation compared to well-fed bucks provided with shade.

1716 - Comparison of Firocoxib and Meloxicam for Pain Mitigation in Goats Undergoing Surgical Castration

Author: Mikaela Weeder , Michael Kleinhenz, Emily Reppert, Leslie Weaver, Ally Leslie, Andrew Curtis, Bailey Fritz, Hans Coetzee

Objectives

Castration is a common husbandry practice in food animal management. Castration reduces aggression, prevents indiscriminate breeding, and improves carcass quality. For male goats intended to be kept as pets, castration is recommended closer to sexual maturity (6-8m) to allow for increased urethral diameter. A larger urethral diameter lowers the chances of ureteral obstruction occurring. There are currently no approved analgesic drugs for surgical castration in goats. Veterinarians must extrapolate analgesic data from other livestock species to try and mitigate pain in goats. Sheep and cattle are commonly used, but there is little research supporting best practices of pain mitigation in goats. This study sought to examine the effects of firocoxib and meloxicam after surgical castration in crossbred goats.

Material and methods

Eighteen male goats, ages 6 to 8 m, were enrolled on study. Twelve of these were castrated. Castrated animals were ran- domly assigned to either a treatment of firocoxib (FIRO, n = 6) or meloxicam (MEL, n = 6). Six male goats served as controls and were kept intact, (CNTL, n = 6) along with being given a placebo treatment of whey protein powder in a gelatin bolus. Treatments were administered at 0, 24 and 48 h. Firocoxib was administered at 1 mg/kg orally prior to induction (0 h), and then at 0.5 mg/kg orally at 24 and 48 h post castration. Meloxi- cam was administered at 2 mg/kg orally prior to induction (0 h), and then at 1 mg/kg orally at 24 and 48 h post castration. All outcomes were taken at –24, 4, 8, 24, 48 and 72 h. Measured out- comes included visual analog scale (VAS), infrared thermography (IRT), plasma cortisol, and kinetic gait analysis (KGA).

Results

The VAS for FIRO goats (51.83 ± 5.24 mm) was significantly higher at 48 h when compared to MEL (35.50 ± 5.24 mm) and CNTL (0 mm ± 5.24 mm) ($P < 0.0001$). There were no significant differences in temperatures for IRT images of the medial canthus of the eye among groups. Mean cortisol values for FIRO (4.87 ± 2.67 ng/mL) and MEL (4.62 ± 2.67 ng/mL) were significantly higher when compared to CNTL at the 8 h timepoint (1.43 ± 2.84 ng/mL). Average rear stride length change from baseline (KGA) for FIRO (-18.24 ± 5.66 cm) was significantly shorter when compared to both MEL (3.76 ± 5.66 cm) and CNTL groups (0.16 ± 5.66 cm) ($P = 0.0324$) when comparing treatment means. Mean values for gait velocity change from baseline values differed between FIRO (-54.17 ± 18.21 cm/sec), MEL (14.54 ± 18.21 cm/sec) and CNTL (-3.06 ± 18.21 cm/sec) ($P = 045$).

Conclusions

Results from this study provide promising evidence that meloxi- cam may provide analgesia after surgical castration. Meloxicam had lower VAS scores, slightly lower mean cortisol values, a longer stride length change from baseline, and a faster gait change velocity from baseline when compared to firocoxib. Refinement of analgesia dosages and castrated control animals are factors that will be included in future research studies. Further evalua- tion of firocoxib and meloxicam administration following surgical castration is needed in this age of goats.

1764 - Prevalence of Small ruminant lentiviruses (SRLV) in goats from a Veterinary Diagnostic and Epidemiology Service in Brazil: a seven-year retrospective study

Author: Nathalie Costa da Cunha, Thalyta Rodrigues Silva, Isadora de Fatima Braga Magalhaes, Carolina Cerqueira Rodrigues, Virginia Léo de Almeida Pereira, Elmiro Rosendo do Nascimento, Mário Felipe Alvarez Balaro

Objectives

Lentiviruses belongs to a group of retroviruses that includes those affecting small ruminants with a pivotal role in health and animal productivity. Epidemiological studies are crucial for understanding the prevalence and transmission dynamics of small ruminant lentiviruses (SRLV). It also provides valuable insights for the development of effective control measures. This study proposed to survey cases of SRLV in goats diagnosed in the last seven years from samples received by the Veterinary Diagnostic and Epidemiology Service in Fluminense Federal University, Brazil.

Material and methods

Blood samples were received in the Veterinary Diagnostic and Epidemiology Service from veterinarian practitioners for molecular diagnosis of SRLV. This veterinarian also filled out a short questionnaire (made by our epidemiology service) about the production system when sending samples. All of them came from goat flocks displaying clinical signs such as weight loss, arthritis, posterior paresis, ataxia and mastitis. For SRLV diagnosis, it was carried out polymerase chain reaction (PCR) with specific primers for the gag gene, using the leukocyte coat from blood samples. Amplification products were confirmed by electrophoresis on 1.5% agarose gel stained with ethidium bromide by detection of a 187-bp fragment. Cases from 2016 to 2023 were considered, even though no data from 2020 to 2021 were obtained due to COVID pandemic.

Results

It was received 619 samples from four Brazilian states, being 544 from Rio de Janeiro (RJ), 29 from Minas Gerais (MG), 11 from São Paulo (SP) and 35 from Paraná (PR). A total of 19 goat farms being 11 from RJ (Flocks A to K), four from MG (Flocks L to O), three from SP (Flocks P to R), and one from PR (Flock S) were sampled. The overall prevalence by PCR was 27.5% (170/619) for SRLV. Over years, the prevalence for SRLV was 57.9% (33/57), 7.7% (1/13), 30.2% (45/149), 18.7% (20/107), 30.8% (54/175), 14.4% (17/118), in 2016, 2017, 2018, 2019, 2022, and 2023, respectively. The prevalence of positive animals for SRLV in each flock was: A (9.9%; 14/142), B (7.7%; 1/13), C (12.2%; 6/49), D (53.8%; 50/93), E (66.2%; 51/77), F (40.0%; 2/5), G (37.5%; 18/48), H (7.8%; 5/64), I (50.0%; 1/2), J (20.0%; 1/5), K (10.9%; 5/46), L (20.0%; 4/20), M (0%; 0/1), N (71.4%; 5/7), 0 (0%; 0/1), P (50.0%; 1/2), Q (0%; 0/1), R (50.0%; 4/8), and S (5.7%; 2/35). The prevalence of positive animals per state was 28.3% (154/544) in RJ, 31.0% (9/29) in MG, 45.5% (5/11) in SP, and 5.7% (2/35) in PR. From samples received, there were clinical description of thin animals (body condition score ≤ 2) and polyarthritis (carpal and tarsal regions), although no neurological signs. Regarding the production system, all goat flocks have the following management in common: kid segregation after birth, controlled or natural mating system and entry of animals into properties through exchange or purchase of breeding stock.

Conclusions

The outcomes unveil a notable prevalence of SRLV among goat flocks distributed across various states of Brazil. Considering that some animals displayed no clinical signs, this underscores the importance of SRLV diagnosis even in cases of asymptomatic individuals, emphasizing the need for further comprehensive epidemiological studies.

1813 - Typology of goat production systems in the Southern Highlands of Peru

Author: Emmanuel Alexander Sessarego Dávila, Walter Palomino Guerrera, David José Godoy Padilla, Juancarlos Alejandro Cruz Luis, Fritz Carlos Trillo Zarate

Objectives
This study aimed to typify goat production systems in the Southern Highlands of Peru.
Material and methods
A structured questionnaire was developed and interviews were conducted with 168 goat smallholders in three districts from Ica and four districts from Ayacucho, regions of Peru, located at an altitude from 2500 to 3800 m. Once the data was collected, 10 categorical variables were selected such as region, district, education level, farmer decision-making, main farming activity, husbandry purpose, mixed farming, feeding practice, land tenure, and milk production. A Multiple correspondence analysis (MCA) followed by a hierarchical classification analysis (HCA) was performed with the FactoMineR package from R software (version 4.3.2).
Results
Results of typology revealed four types of goat production systems that were mainly differentiated by region, district, and feeding practice. Type 1 included goat smallholders from Ayacucho, who had completed elementary or middle school, decision-making of livestock was taken by the wife, crop production was the main activity, there was no mixed husbandry, goats fed by a grazing system in communal areas, and with milk production/doe of one liter per day, mainly for self-consumption. Type 2 was determined by farmers mainly from the district of Ocaña, Ayacucho, where they feed their animals in communal areas, being animal husbandry as the main economic activity. Type 3 was characterized by including farmers from the districts of San Pedro de Hucarpana and Chavin, Ica, where their principal incomes were obtained through dairy products, with crop and livestock being the main farming activities. Finally, type 4 included goat farmers from the district of San Juan de Yánac, Ica, which use their lands and, often, rented lands for husbandry, and the feeding practices of goats were grazing crop stubble and natural pastures.
Conclusions
In conclusion, in the Southern Highlands of Peru, there four types of goat production systems with a common characteristic of being extensive farms with low resources and benefits; which require public policies that establish strategies that allow for improving animal productivity and, with it, sustainable development of goat farming.

1821 - Characterization of Extensive Goat Production Systems in Climate-Vulnerable Dry Forests in Peru: A Comprehensive Analysis

Author: Victor Alexander Temoche Socola

Objectives
The objective of the study was to characterize the components of the goat production system located within the dry forests of northern Peru and to assess the relationship between the levels of these components and the producer's perception of climate change
Material and methods
A total of 130 goat livestock producers were interviewed, and sociocultural, productive, economic, and environmental variables were analyzed using descriptive and multivariate statistics (PCA, MCA, hierarchical and two-step clustering). In addition, the multiple correspondences of the variables of each component with the perception of the climatic change of the producers were evaluated.
Results
From this, it was derived that livestock farming operates in an extensive system, represented by 84.62% of producers. The average populations consist of 85 animals per producer, grouped in mixed herds of Creole cattle, situated in uncategorized facilities. Feeding is based on the floristic composition of the 76 plant species in the dry forest, distributed among the arboreal stratum (14%), shrub stratum (21%), and herbaceous stratum (65%). Only 40.77% use stubble from the area, and 25.4% have chosen to supplement their animals. Reproduction occurs through natural mating, and only 92.3% administer doses to their animals.
The classification analyses revealed that, for the sociocultural component, there is a prevalence of high sociocultural condition (55.4%) over low sociocultural condition (44.6%). Regarding the productive component, three classes were identified: high production capacity (33.85%), medium production capacity (51.53%), and low production capacity (14.62%). In the economic component, there is a presence of producers with high economic condition (50.8%) and producers with low economic condition (49.2%). For the environmental component, there are producers with excellent adaptation capacity (6.16%), good adaptation capacity (23.08%), regular adaptation capacity (75.38%), and poor adaptation capacity (24.62%). Finally, in the ecological component, the perception of the forest condition resulted in good (0%), bad (83.1%), and equal (16.9%). 56.9% of producers acknowledge climate change; however, 79.2% attribute it to environmental pollution. Climate change is perceived through temperature variations (69.9%), changes in precipitation (100%), soil productivity impact (79.2%), and water availability reduction (50%).

Conclusions
It is concluded that producers have greater chances of continuing developing the goat system and their perception of climate change improved if they have greater access to training, education and associativity.

SHEEP

1014 - Influence of heat stress on body temperatures measured by infrared thermography and blood metabolites in sheep

Author: Radojica Djokovic, Marko Cincovic, Aleksandar Cukic, Milos Petrovic, Biljana Andjelic

Objectives

The aim of this research is to examine the influence of heat stress (HS) on body temperature (BT) measured rectally (RT) or by infrared thermography (IRT) of the nose (NT), eye (ET), leg (LT) and abdominal (AT) regions in intensively and extensively breed sheep and to detect a correlation between body temperature and metabolic response in sheep.

Material and methods

A total of 33 Wurttemberg × Sjenica Pramenka sheep breeds were examined, 17 ewes were from outdoors and 16 were from indoor housing systems during three experimental periods (thermo-neutral period, severe HS and moderate HS). Temperature-humidity index (THI) were measured three times a day in three different periods of the experiment, at 07:00 (THI morning), 14:00, (THI day) and 19:00 (THI night). Rectal temperature was measured by a standard digital thermometer. The body surface temperature was measured in all animals by collecting images in different parts of the body-thermal window: eye temperature (ET), nose temperature (NT), front leg temperature (LT) and abdomen temperature (AT). Thermographic images were obtained with an infrared camera, Testo 865 (Titisee-Neustadt, Germany). Thermal images of the eyes and nose were obtained at a distance of approximately 20 cm, and the front leg and the abdomen images at a distance of less than 1 m. Thermal imaging was performed in three different periods of the experiment, in a time interval of 12:00–16:00 h, simultaneously with blood sampling. Blood samples were collected by jugular veni puncture, for analysis: cortisol, triiodothyronine (T3), thyroxine (T4), insulin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), glucose, calcium (Ca), inorganic phosphates (P), magnesium (Mg), total protein (TP), albumin, globulin, urea, triglycerides (TG), cholesterol (Chol.), total bilirubin (TBil.), creatinine (CR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatine kinase (CK). Standard kits from Randox (Crumlin, UK) for NEFA and BioSystem (Barcelona, Spain) for other parameters were used on Rayto Chemray 120 spectrophotometer (Rayto Life and Analytical Sciences, Shenzhen, China). An automated immunoassay analyzer, TOSOH AIA-360 (Tosoh Bioscience, Tokyo, Japan), was used for endocrinological analyses. Statistical analysis included the use of GLM and ANOVA analysis to examine the effect of period, breeding method and period × housing method interaction on the value of body temperature measured rectally or by thermography of different body parts and blood biochemical parameters. The connection between parameters were examined, using the Pearson correlation coefficient (SPSS statistics software , IBM, Armonk, NY, USA).

Results

Sheep under HS have a higher BT ($p<0.05$) than sheep during thermo-neutral period. The magnitude of BT measured by infrared thermography (IRT) was higher ($p<0.05$) than RT. A higher RT ($p<0.05$) is found in sheep under indoor housing, but the temperature obtained by IRT is significantly higher ($p<0.05$) in sheep under an extensive breeding system (significant effect of breeding). LT and AT showed positive linear correlations ($p<0.01$) with the THI, while other ways of measuring BT did not give statistically significant correlations ($p>0.05$). Sheep under HS showed higher ($p<0.05$) blood cortisol, insulin, TP, albumin, urea, CR, Tbil., AST, ALT, GGT, ALP, LDH, and CK, with lower ($p<0.05$) values of T3, T4, NEFA, BHB, glucose, Ca, P, Mg and Chol. BT and metabolic response were different in the function of the housing method of sheep. LT and AT showed a significant correlation ($p<0.05$) with almost all blood parameters, and the strongest connections ($p<0.01$) were made with T3, T4 and BHB. The abdomen and legs are good thermal windows, because LT and AT are good summative responses to external ambient THI and internal metabolic changes in sheep under heat stress.

Conclusions

Under the influence of HS, body temperature increases in sheep, and this increase is more pronounced in extensively reared animals. Metabolic changes caused by HS are equally pronounced in sheep in intensive and extensive breeding. The use of a thermal imaging camera in the assessment of heat load in sheep show great advantages over rectal temperature measurement due to its sensitivity to ambient conditions. The temperature of the abdomen and front legs significantly correlates with the THI value but also with the values of metabolic parameters, so it could be a suitable method in the non-invasive assessment of stress load in sheep, especially in extensive sheep breeding.

1036 - Caseous Lymphadenitis in Sheep: A Case Study and Management Model in Morocco

Author: Tarik Embarki, Pedro Rodriguez Fernandez

Objectives

Sheep caseous lymphadenitis, caused by *Corynebacterium pseudotuberculosis*, is a significant concern for sheep farmers worldwide. This study aimed to test the effectiveness of tildipirosin on caseous lymphadenitis and monitor the outcome of cases after treatment.

Material and methods

The study was conducted at a sheep farm with 1600 heads located in the Khmissat region, 130km from Rabat (Morocco). The farm had a history of prevalence of the disease for over six years.

A total of 88 cases were included in the study, with recorded information on age, gender, breed, abscess location and size. Abscess sizes were evaluated using a scoring system from 1 to 5 with 1 (<2 cm), 2 (2-5 cm), 3 (5.1-10cm), 4 (10.1-15 cm) and 5 (>15 cm). On day 0, all clinical cases were treated with a single injection of tildipirosin, a semi-synthetic macrolide antibiotic, indicated for the treatment of bovine respiratory disease in cattle. Based on previous studies that evaluated pharmacokinetic characteristics in sheep, a dose of 4 mg/kg body weight (1 mL/45 kg) was chosen. All animals were monitored for abscess location and size at day 30 and 60 after initial treatment. Descriptive analysis and statistical methods were employed to analyze the data using Excel and IBM SPSS STATISTICS version 23. Prevalence calculations and comparisons were performed using the Chi-square test for age, sex, position, and size of abscesses.

Results

An initial prevalence of 15% (240 animals) was recorded in the herd, however, only 88 animals were included in the study. Disease prevalence was 41% in females and 59% in males. Irrespective of the animal age, the submandibular region accounted for 41% of the abscesses, followed by the prescapular lymph node (19%) and the parotid lymph node (18%). Further analysis showed that in young animals, 44% had abscesses in the submandibular region, while 35% had them in the parotid region. For adults, 39% of the abscesses were in the submandibular region and 25% in the axillary region. Regarding the abscess size, 17% of the abscesses had a score of 1, 40% score of 2, 28% score of 3, 10% Score of 4 and 5% Score of 5. Old abscesses were more common in adult animals, while the size and location of abscesses varied based on the age of the animals: 35% of young animals had parotid abscesses whereas 25 % of the adult animal's abscesses were found in the prescapular region, suggesting that bacterial introduction may occur through injuries to the arms and lower neck during grazing in adults while in younger animals, micro-injuries are likely to occur in the oral cavity. An overall recovery rate of 87% was observed after treatment, with 38% showing complete recovery and 47% showing partial recovery (reduction in size but not total elimination) by day 30. On day 60, 91% of young animals and 84% of adults had completely recovered without significant difference between the two groups (p=0.356). Interestingly, healing was faster in new versus old abscesses regardless of their size.

Conclusions

The results demonstrated the efficacy of tildipirosin as a treatment option for caseous lymphadenitis. The study also suggests a successful management of caseous lymphadenitis by proper identification, recording and treatment of clinical cases. In addition, the study provides an important insight into the disease prevalence in relation to the affected sites on the body, type of season, and age of animals.

1085 - Establishing baseline mastitis parameters on commercial New Zealand sheep milking farms

Author: Greg CHAMBERS, Matthew WELLS

Objectives
Sheep milking is a rapidly growing and dynamic industry in New Zealand. Sheep farmers have identified mastitis as a research priority because it is a disease that causes significant losses but is also one for which baseline New Zealand data are lacking. Decisions on farms and at an industry level are, therefore, often based on overseas evidence and recommendations, as veterinarians and farmers lack systematically collected New Zealand-based information to make evidence-based decisions. To address these challenges, the large-scale study summarised here was launched to describe basic mastitis parameters for New Zealand dairy ewes and explore relationships between tests and risk factors for mastitis.

Material and methods
Over the 2022-2023 season, a prospective multi-pronged study was run on 20 commercial dairy sheep farms spread across New Zealand. It collected general farm information and data on clinical and subclinical mastitis, individual ewe and bulk milk somatic cell count (SCC), the bacteriology of clinical and subclinical mastitis cases, udder and teat conformation, and health, body condition score, and risk factor information. It has also recorded how farmers manage and treat mastitis, captured mastitis outcomes, and will be used to develop tests for accurately identifying mastitis, especially subclinical mastitis, and assess how herd-level test results reflect mastitis at the individual ewe level.

Results
The study is still ongoing, but preliminary findings are available. Clinical mastitis appeared to occur around lambing predominantly and was dominated by environmental bacteria (particularly *Streptococcus uberis*), *Staphylococcus aureus*, and non-aureus staphylococci. SCC of randomly sampled ewes across three time points had a median of 131,000 and a mean of 948,562 cells/ml, with 11% of ewes having an SCC >1,000,000 cells/ml. The rapid mastitis test correlated well with SCC performed on the same milk sample. Non-aureus staphylococci were the most prevalent microbes isolated from randomly sampled ewes without clinical mastitis.

Conclusions
Baseline mastitis parameters are being established for New Zealand dairy sheep, and tools such as SCC and the rapid mastitis test are being validated. Risk factor information will also be collected. The results will be updated as the study progresses. The findings will be used to guide farm-decision makers and advisors on how to manage mastitis on sheep milking farms, informing a long-term journey of improvement and increasing the sustainability and profitability of sheep milking in New Zealand.

1091 - The influence of selenitetriglycerides on the health status and expression of selected genes encoding enzymes involved in antioxidant processes in small ruminants.

Author: Przemysław Sobiech , Karolina Tatarczak, Dawid Tobolski, Jan Miciński

Objectives

Selenium deficiency is still a current problem in both sheep and goat farms. It may be one of the causes of disorders of the immune system, reproductive system and diseases, including nutritional muscular dystrophy. The organic form of selenium that can be orally administered to animals is selenitetriglycerides. This substance is a new form of selenium and characterized by low toxicity, a fairly high bioavailability and a high level of absorption from the gastrointestinal tract

Material and methods

The research was carried out on 12 lambs of the Kamieniecka sheep and 12 kids of the Alpine goat, aged 10 days. The animals were divided into 2 groups: control and experimental. For the first 7 days of the experiment, the experimental group was orally supplemented with a solution of selenitetriglycerides, at a dose of 0.5 mg selenitertriglicerydes, per kg of body weight. Then, blood was collected from all animals on the 0th (before administration of the selenitetriglyceride solution for the experimental group), 1st, 7th, 14th, 21st and 30th day of the experiment. On the day 30. of the experiment, lambs and kids from both groups were euthanized, then the samples of the longisimus dorsi muscle, biceps femoris muscle, myocardium and liver was taken from them. The following parameters were estimated in the blood serum: concentration of glucose, albumin, total protein, creatinine, urea, activity of aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT) and lactate dehydrogenase (LDH) activities and level of selenium. In addition, the activity of the glutathione peroxidase (GPX) and superoxide dismutase (SOD) in whole blood of all animals, were determined. In the tissue samples of biceps femoris muscle, myocardium and liver in kids and lambs, and longissimus dorsi muscle in lambs the analysis of mRNA expression of selected genes encoding enzymes involved in antioxidant processes, i.e. GPX1, SOD1, was determined.

Results

AST activity of lambs on the day 1 of the experiment was significantly lower in the experimental group and activity of GGT in kids was significantly higher ($p \leq 0.05$) in the control group on the days 7, 21 and 30. The 30th day of the experiment also showed a significantly ($p \leq 0.05$) higher AST activity in the kids of the experimental group.

The results of serum selenium concentration in lambs and kids showed that supplementation with selenintetriglyceride causes a significant ($p \leq 0.05$) increase in selenium concentration on the 1st, 7th, 14th, 21st and 30th days of the experiment. The highest selenium concentration in the experimental group was recorded on day 7 of the experiment for both lambs and kids and reached 464 and 509 µg/l respectively.

In addition, significantly higher ($p \leq 0.05$) GPX activity was observed in kids on the 1st, 7th, 14th, 21st and 30th days of the experiment. Similarly, SOD activity was significantly higher ($p \leq 0.05$) in selenitetriglyceride-supplemented kids. Moreover, GPX and SOD activities was significantly higher in in the lambs from supplemented group.

After supplementation with selenitetriglycerides, an increase in the expression of the GPX1 gene in the biceps femoris muscle, liver in kids was observed. Moreover an increase in the expression of the SOD1 gene in the analyzed fragments of the liver in kids, as well as in the longissimus dorsi muscle (m. longisimus dorsi),, liver and myocardium in lambs was noticed. In the case of the longisimus dorsi muscle, myocardium and liver in lambs, as well as the heart muscle in kids, a decrease in the expression of the GPX1 gene was observed. Similarly, the SOD1 gene expression was decreased the biceps femoris muscle (m. biceps femoris) of lambs and the biceps femoris muscle muscle and the heart in kids. In the case of the biceps femoris muscle in lambs, the expression level of GPX1 remained at a level comparable to the control group.

Conclusions

Selenitetriglycerides cause a significant increase in the concentration of selenium in the body of supplemented small ruminants and have not a negative effect on the results of biochemical parameters in lambs and kids, and therefore can be used as a safe source of selenium supplementation in their organisms. Supplementation with selenitetriglycerides did not significantly affect the expression of GPX1 and SOD1 genes.

1106 - Comparison of post-thawing/warming viability of in vitro produced ovine blastocysts cryopreserved by slow freezing and vitrification.

Author: Zaira Paola Gonzalez Gomez, Horacio Álvarez-Gallardo, David Urbán-Duarte, Adriana Velázquez-Roque, Francisco Javier Padilla-Ramirez

Objectives

The objective of this research was to evaluate the viability of post-thawed/warmed *in vitro* produced ovine embryo by effect of two cryopreservation methods: slow freezing and vitrification.

Material and methods

2.1 In vitro embryo production (IVP)

For IVP, IVF-Bioscience™ media were used for *in vitro* maturation (BO-IVM™), *in vitro* fertilization (BO-IVF™) and *in vitro* culture (BO-IVC™).

2.1.1 In vitro maturation (IVM)

Ovaries were collected from a slaughterhouse and transported (37°C) to the laboratory within 2 h in saline solution (0.9% NaCl) supplemented with penicillin G (100 IU/mL) and streptomycin sulfate (100 µg/mL). For the IVM, the cumulus-oocyte complexes (COCs) were selected (only grades 1 and 2) and matured for 24 h at 38.5°C in 5% CO_2 in air and 100% humidity.

2.1.2 In vitro fertilization (IVF) and culture (IVC)

For IVF, frozen-thawed semen from an IVP proven ram was used. Matured oocytes were fertilized *in vitro* with semen adjusted at a concentration of 2 x 10^6 sperm/mL and placed with oocytes for 18 h in 38.5°C, 5% CO_2 in air and 100% humidity. Presumptive zygotes were denuded by pipetting and set in IVC until day 7 at 38.5°C, 5% CO_2, 5% O_2 and 90% N_2 at 100% humidity.

2.2 Cryopreservation

Expanded blastocyst stage embryos (n=300) quality 1 and 2 (according to the fourth edition of the International Embryo Transfer Society Manual) were divided in 3 groups (100 each): Control (Cx), cryopreserved by vitrification (VIT), and slow freezing (SL).

2.2.1 Slow freezing

Blastocysts were subjected to a controlled-rate freezing curve (CL-8800 Cryologic™, Australia) after equilibration for 8 to 10 min in freezing medium with EG (Ethylene Glycol Freeze Plus Vigro™), starting in -6°C (seeding) and decreasing 0.5°C/min, and ending at -35°C and then plunging directly into liquid nitrogen.

2.2.2 Vitrification

Blastocyst were vitrified in a protocol with EG and DMSO. Embryos were immersed in vitrification solution 1 (PBS, 10% BSA + 7.5% EG + 7.5% DMSO) for 5 min and then into vitrification solution 2 (PBS, 10% BSA + 15% EG + 15% DMSO + sucrose 0.5 M) for 40 s. Embryos were loaded into Cryotop device (Kitazato™) and stored in LN2.

2.2.3 Thawing/warming.

The embryos (SL and VIT) were thawed/warmed. Each group were divided equally to evaluate re-expansion, hatching rates, and viability with fluorescent staining.

2.3 Embryo viability evaluation by fluorescent staining

Cx, thawed, and warmed embryos were double-stained to assess total cell number and number of cells with an altered cell membrane. Embryos were incubated for 15 min in a protein-free maintenance solution supplemented with 10 µg/mL propidium iodide (PI),

fixed in 70% ethanol for 5 min, and transferred into ethanol containing 10 µg/mL Hoechst 33342 for 5 min, stained embryos were placed in a glycerol droplet on a glass slide and covered with a coverslip, examined under fluorescence microscopy at 460 nm. Hoechst positive fluorescing blue (viable cells) and PI positive fluorescing red (non-viable cells).

2.4 Statistical analysis

The response variables were re-expansion and hatching rates at 24 and 48 h, total cells, viable cells, and non-viable cells for each treatment. Statistical analysis was carried out using the Chi-squared (for re-expansion and hatching) and ANOVA (for total cells, viable cells, and non-viable cells) procedures on the Jamovi software (version 1.2; The Jamovi project, Sidney, Australia).

Results

The results obtained for viable cells (180.10 ± 7.885, 112.42 ± 3.328 and 91.88 ± 6.325) for the Cx, SL, and VIT group respectively were significant differences between the groups. In the case of non-viable cells there were no difference between the Cx and SL group, however, both groups had significant differences with respect to the VIT group. Regarding the number of total cells, there was no significant difference ($P>0.05$) for the Cx and VIT group (185.04 ± 7.585 and 175.26 ± 5.386), contrary to the frozen (121.54 ± 3.258), there were significant differences ($P<0.001$) between both groups.

Conclusions

In conclusion, under the conditions of this research, embryos subjected to slow freezing had better post-thaw viability compared to embryos subjected to vitrification, showing alternative with promising results for the long-term conservation of *in vitro* produced ovine embryo.

1261 - First identification of ovine neosporosis from an aborted fetus in Serra da Estrela sheep, Portugal, 2023.

Author: Rita Cruz, FERNANDO ESTEVES ALMEIDA, Helena Vala, João Mesquita, Sara Gonçalves, Carla Santos, Cristina Mega, Carmen Nobrega, Maria Pereira, Abigail Barbosa, Carla Afonso

Objectives

Neospora caninum is a protozoan parasite that affects dogs and cattle. Although this pathogen is less commonly seen and less well-documented in small ruminants, few data highlight its role as an abortive agent in sheep. Hence the major objective of this work is to study the role of congenital neosporosis in reproductive losses in dairy sheep of Central Portugal region.

Material and methods

From a sheep farm that consisted of 167 Serra da Estrela autochthonous sheep, 17 sheep experienced abortions at various gestational ages. From the 11 aborted fetuses that were observed in the field, two were further studied by searching for pathogens likely to be responsible for abortions by quantitative polymerase chain reaction (qPCR). Additionally, serological screening for anti-*Neospora caninum* was also performed in 8 adult sheep aborted.

Results

From the two aborted fetuses studied by qPCR, both shown to be positive for *Neospora caninum* DNA, with cycle thresholds of 26.4 and 28.6, respectively. Both fetuses were negative for the qPCR screening for Chlamydia, Coxiella, Listeria and Pestivirus DNA. From the eight sera tested for anti-*Neospora caninum*, four (50%) showed to be positive for antibodies.

Conclusions

Results from this study highlight the need to consider *Neospora caninum* as an abortive agent in sheep and alert for the need to control the interface between dogs and sheep in this region.

Acknowledgments: This work is supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the projects CITAB UIDB/04033/2020, CERNAS UIDB/00681/2020 and GHTM UID/04413/2020.

1358 - The effect of combining melatonin and progestogens sponges in Assaf ewes for out of season breeding

Author: Javier Acosta Ledesma, Carlos M. Bursa, Federico Randi

Objectives

Melatonin is synthesized in the pineal gland and transfers the day/night signals to the reproductive neuroendocrine axis.

Melatonin implants have been widely used to advance the breeding season of anoestrous ewes and goats. They induce high plasma concentrations of melatonin for 24h every day, without suppressing the endogenous secretion of the pineal hormone during the night.

The addition of melatonin implants to protocols of progestogens sponges+eCG for out-of-breeding season should have the ability to maximize the flock pregnancy rates and reduce the lambing spread of the treated ewes. This study tested the hypothesis that administration of melatonin implant one month before synchronized mating improves fertility performances of Assaf ewes.

Material and methods

552 mature Assaf ewes were synchronized using either a 7-day or a 14-day progestogen-based program. The treatments started in the month of April. The animals were located in the Palencia province, in the North of Spain. On 300 ewes a melatonin implant (Melovine® 18mg, natural melatonin, Ceva Sante Animale, Libourne, France) was administered at the base of the ear, 34±2 days before the mating start date. This group was defined Melovine®+Sponges (n=300). The remaining animals (n=222) did not receive the implant and were defined as group Sponges. All the ewes 16 or 9 days before mating start date received a 30–mg fluorogestone acetate (FGA) vaginal sponge (Synchro-part® 30 mg, Ceva) and at sponges removal received the injection of 480 IU of equine chorionic gonadotropin (eCG) (Synchro-part PMSG®, Ceva). Males were introduced to the ewes 48h after the sponges removal and kept for 40 days. Pregnancy establishment was evaluated by transabdominal ultrasonography 30 to 40 days after rams removal. Data were analyzed using a non-parametric Fisher exact test for two proportions.

Results

The application of Melovine® »30 days before rams introduction in synchronized Assaf ewes, increased the fertility of the treated animals.

The 84% of Melovine®+Sponges ewes exposed to rams, became pregnant (252/300) whereas only 65% of the ewes, which did not receive the melatonin implant, Sponges group, established a pregnancy (145/222), ($p < 0.00001$).

Conclusions

The administration of Melovine® 30 days before ram introduction in Assaf ewes synchronized with 7 or 14-day protocols of FGA sponges+eCG significantly increased the pregnancy establishment out of the breeding season.

1426 - EFFECT OF DIFFERENT BREEDING SYSTEMS ON EWE PREGNANCY RATE AND LAMB PERFORMANCE

Author: Cristina Sotomaior Santos, Caroline Santos, Bernardo Nogueira, Diógenes Santana, Matheus Carvalho, Saulo Weber

Objectives
The seasonality of European-breed ewes challenges the development of sheep production under mid-latitude, mainly the production of lamb meat aimed at distributing births throughout the year. Therefore, this study aimed to evaluate the reproductive performance (pregnancy rate - PR) of ewes, from meat breeds of European origin, lactating or weaned, bred in different seasons of the year in a region of mid-latitude. The objective was also to evaluate the effect of birth seasons on performance of lambs born.
Material and methods
Four groups of breeding seasons (BS) (n=12) were evaluated, totaling 48 ewes. BS were performed in two stages: outside the reproductive period (stage 1) and within the reproductive period (stage 2). Furthermore, at each stage, the ewes were separated into two groups: LG, in which BS was performed while the ewes were lactating; and WG, in which BS began after the lambs were weaned. The ewes were monitored, every 2 weeks, with parasitological analyzes (fecal egg count – EPG) and body condition score (BCS) assessments, from the final third of pregnancy, until the end of the next BS. Pregnancy diagnosis was performed by ultrasound, 30 days after the end of the BS, to calculate the PR. The statistical analysis of the PR and the average EPG count of the different groups was carried out using the Chi² method and T Test, respectively. The offspring of these ewes, 62 lambs, were also evaluated, divided into two groups: WG60, weaned at 60 days of age and WG90, weaned at 90 days of age, in two different stages, from August to November 2022 (stage 1, n = 31), and from February to June 2023 (stage 2, n = 31). The lambs were evaluated weekly, through individual assessment of their weights, and fortnightly with parasitological analyzes, through EPG counting.
Results
In stage 1, outside the traditional reproductive season of the sheep, LG1 presented a PR of 25%, lower ($p<0.05$) than the WG1, with a PR of 83.3%. Within the reproductive season, in stage 2, the PR of WG2 was 100%, higher ($p<0.05$) than that of LG2, with 0%. WG PR, considering stages 1 and 2, were statistically similar ($p<0.05$). The ECC ranged from 2.0 to 3.5, with no difference between groups. WG1 and WG2 presented an average EPG of 515±293 and 648±699, respectively, significantly lower ($p<0.05$) than LG1 and LG2, which presented an average EPG of 2526±1740 and 908±854, respectively. In both stages, the WG90 had a higher average weight gain ($p<0.05$), although they had a higher average EPG count. The number of treatments with anthelmintics was higher ($p<0.05$) in the WG60 group, in stage 1, with no difference ($p>0.05$) between the groups in stage 2. In the comparison between the same weaning groups, there was a significant difference ($p<0.05$) for both WG60 and WG90 in stages 1 and 2, with stage 1 presenting a greater number of lambs treated.
Conclusions
It was possible to conclude that BS carried out outside the breeding season may have a similar PR to that carried out during the breeding season, as long as the ewes are not

lactating at the time of the BS. The results also allowed us to conclude that, within the proposed objectives, the WG90 group achieved the best performance.

1479 - ETIOLOGIC PROFILE AND ANTIMICROBIAL SENSITIVITY IN BACTERIAL ISOLATES OF SHEEP MILK – Preliminary data

Author: Andressa Gonçalves , Natália Gaeta, Raquel Raimondo

Objectives
Animal production, including sheep farming, poses a potential risk for the emergence and dissemination of antimicrobial-resistant genes and bacteria, largely attributed to antimicrobials indiscriminate use. Despite this concern, there is limited knowledge about sheep production antimicrobial resistance. This study addresses this subject by focusing on sheep mastitis etiology and antimicrobial susceptibility of the bacterial isolates.
Material and methods
Eighty milk samples from commercial dairy sheep producers in Brazil were collected and evaluated from a total of 370 samples. 30 to 90 days of lactation ewes were selected, and the meticulous milk samples collection process involved antiseptic cleaning of teat holes before sterile milk collection for microbiological evaluation. To comprehensively evaluate milk composition and somatic cell count (SCC), 40 ml of milk were collected in bronopol preservative bottles, with an additional 2 ml for the California Mastitis Test. Microbiological milk samples analysis was conducted using specific culture media, such as blood agar, salted mannitol, and MacConkey agar. Colonies were characterized using MALDI-TOF and preserved for future analysis. Antimicrobial resistance profiles were determined using the disk-diffusion test with major antimicrobial classes prescribed in veterinary and human medicines. Future molecular identification involves PCR for mecA and mecC genes in isolates resistant to cefoxitin (classified as methicillin-resistant), and for the blaCTX-M gene in bacteria producing extended spectrum beta-lactamase.
Results
Eighty samples have been collected from 40 dairy sheep, with 52 showing positive bacterial isolation. Notably, eight microbiologically positive samples exhibited the presence of two different bacterial species, suggesting a complex microbial landscape within the flock. Thirty-six Staphylococcus species were submitted to the antibiogram test and three species of eleven samples presented resistance. Four Staphylococcus epidermidis samples (66,66%) were Penicillin resistant and two (33,33%) Tetracycline resistant, whereas one was resistant to both molecules. One of four Staphylococcus haemolyticus samples was Penicillin resistant, totaling 25%. A comparative analysis revealed changes in milk composition and SCC in bacterial isolation samples, including decreased lactose concentration and increased total protein, casein, and somatic cell count.
Conclusions
Upon project completion, we anticipate obtaining valuable insights into mastitis etiology in dairy sheep production, mapping the frequency of antimicrobial-resistant bacteria and associated resistance genes, and identifying potential management deficiencies that are promoting spreading and selection of resistant microorganisms. Ultimately, our findings aim to enhance understanding and directly impact dairy sheep production sector, contributing to antimicrobial resistance reduction.

Keywords: antimicrobial resistance, mastitis, dairy sheep farming.

1498 - Mandibular Osteomyelitis in Sheep caused by Pseudomonas aeruginosa

Author: Helio Martins de Aquino Neto, Irma Ximena Barbosa Sanchez, José Alexander Correa Díaz, Sergio Fabian Quebrada Molina

Objectives

Osteomyelitis is a bone inflammation, localized or generalized, often caused by bacterial infections in animals. Trauma or injuries in the oral cavity, caused by ingested food, can be predisposing factors for infectious conditions by opportunistic bacteria. Among these agents, *Actinomyces bovis*, *Actinobacillus lignieresii*, and other bacteria such as *Listeria monocytogenes*, *Nocardia* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, and *Arcanobacterium pyogenes* are highlighted. The objective of this work is to report a case of mandibular osteomyelitis in a sheep caused by *P. aeruginosa*.

Material and methods

Clinical care was provided to a four-year-old male sheep of the Santa Inês breed, managed in an extensive production system with pasture and mineral salt-based feeding, originating from a production in the Tolima state, Colombia. Progressive weight loss, acute respiratory signs, and the presence of swelling in the jaw were reported. Upon physical examination, apathy, low body condition, nasal discharge, and dyspnea were observed. Additionally, there were firm swelling increases in both jaws, sensitive to palpation, with progressive growth over the last 5 months before clinical evaluation. To assist in the diagnosis, the animal underwent a lateral-oblique radiographic view of the affected mandible. Based on radiographic findings and the patient's clinical condition, the owner opted for euthanasia. Upon cross-section of the mandibula, a firm bony mass with purulent secretion inside was identified. This secretion was collected using a sterile swab, transported to the laboratory in thioglycolate broth for etiological diagnosis. The sample was cultured on blood agar and McConkey agar and incubated at 37°C for 24 hours.

Results

The mandibular radiograph revealed bilateral loss of bone density, delimited by the bony structures surrounding the apices of the molars. Necropsy identified rhinitis with caseous necrosis of the ventral nasal concha. Bacterial growth of *Pseudomonas aeruginosa* was observed in the microbiological culture of the collected sample. Microscopically, encapsulated rounded to oval structures, 2 to 3 mm in diameter, with a mixed inflammatory infiltrate consisting of a moderate population of neutrophils, macrophages, and lymphocytes, with central presence of caseous necrosis and bacterial colonies were observed. Peripheral to the structures, there was connective tissue deposition and abundant granulation tissue with moderate angiogenesis and a stroma composed of proliferative fibroblasts and some hemorrhagic areas. The local pain observed during palpation of the mandibular lesion likely compromised the animal's ability to feed, leading to the observed emaciation. *Pseudomonas aeruginosa* is an opportunistic pathogen that typically requires a failure or change in the animal's defenses to cause an infection. The main predisposing factors are periodontal, gingival, and oral mucosal infections, often resulting from injuries caused by ingested food or misuse of dosing guns, serving as an entry point for opportunistic bacteria that are part

of the ruminant oral microbiota. Water sources contaminated with the bacterium are also considered by some authors as a risk factor, although probably less important than oral cavity injuries. There are literature reports of cases of purulent rhinitis caused by *P. aeruginosa* in sheep; however, in the mentioned case, it was not possible to determine if the bacterium was also involved in the etiology of the rhinitis identified during necropsy.

Conclusions

Pseudomonas aeruginosa should be considered as a possible cause of mandibular osteomyelitis in sheep and included as a differential diagnosis for actinomycotic granulomas in this species. Due to its reserved prognosis, preventive measures are essential to minimize the risks of oral cavity injuries, primarily through the provision of higher-quality food, as well as proper training of employees to use syringes and oral dosing guns correctly.

1499 - Evaluation of the administration of intravaginal GnRH for the induction of estrous and ovulatory activity during seasonal anestrus in sheep

Author: Zurisaday Santos-Jiménez, Juan Manuel Guillen-Muñoz, Angela Nakafeero, Martha Isabel Grajales-Bayarri, Paula Martinez-Ros, Antonio Gonzalez-Bulnes

Objectives
The objective of this research was to evaluate the reproductive response (estrus and ovulation induction) to the intravaginal administration of a GnRH analogue (OvuGel®) concomitantly with progesterone during seasonal anestrus.
Material and methods
The research was carried out at seasonal anoestrus (June) at the experimental farm of the CEU Cardenal Herrera University located in Valencia, Spain, at a latitude of 39°N. The procedures used in this research were evaluated and approved by the Animal Experimentation Ethical Committee of the CEU Cardenal Herrera University (report CEEA17/019). Twenty-seven anovulatory Segureña breed multiparous ewes from 2 to 4 parturitions, aged 2 to 6 years and with a body condition of 3.5±0.8, were used. All of them were treated with a controlled-dose intravaginal device (CIDR® Ovis, Zoetis, Madrid, Spain) for 5 days plus an intramuscular dose of $PGF_{2\alpha}$ (5 mg of Dinolytic, Zoetis, Madrid, Spain) at the time of CIDR removal. On day 0 (day of CIDR withdrawal), ewes were divided into 3 experimental groups: first eCG group (n=9) was treated with 300 IU of eCG (Foligon®, MSD Animal Health, Madrid, Spain); second group OVG24 (n=9) and third group OVG36 (n=9) were treated with a vaginal gel containing a GnRH analogue (0.1 mg triptorelin; OvuGel® Vetoquinol, S. A. Lure, France), at 24 and 36 h after CIDR withdrawal, respectively. In all the ewes, the percentage and time of onset of estrus signs (h) were evaluated with trained rams, every 6h from 24h after removal of the CIDR. The percentage and time of ovulation onset (h), as well as the ovulation rate on day 11 of the induced sexual cycle, was performed by transrectal ultrasonography (Eco 3, Chison Co., Jiangsu, China). The comparison of the variables between treatments was performed by analysis of variance (ANOVA), and a Tukey contrast test using the R program version 4.0.5.
Results
The results indicate that the group treated with eCG showed 100% of ewes in estrus. A significantly higher percentage than in the OVG24 and OVG36 groups (44.4 and 66.6%, respectively; $p<0.05$), which showed a later estrus onset of (53.7±7.2 for OVG24; 52.9±5.4 for OVG36 and 39.9±2.2 for eCG; $p<0.05$ for eCG vs the other two groups). The time of ovulation was, however, similar (around 70h) in the three groups. There were no differences in the percentage of ewes bearing corpus luteum on Day 11 after device removal (88.88%, 8/9 for eCG; 66.66%, 6/9, for OVG24 and 77.77%, 7/9 for OVG36).
Conclusions
In conclusion, the use of OvuGel® induces ovulation in treated females but without signs of estrus. Hence, it is not useful for natural mating but, after refinement, may be used for fixed-time artificial insemination. Therefore, further research is needed with a high number of treated females and refinement of the protocol.

1539 - PROGRESS OF A PAG10 CAPTURE ELISA SYSTEM FOR THE DIAGNOSIS OF EARLY PREGNANCY IN SHEEP

Author: Griselda Valdez Magaña , Rogelio Alonso-Morales, Vianey Adoney, Amanda Gayoso-Vazquez

Objectives

Develop an ELISA system for the diagnosis of early pregnancy in sheep using synthetic genes, DNA vaccines, recombinant proteins and monospecific polyclonal antibodies

Material and methods

1) Synthetic genes: The sequence of the PAG10 gene (PAG10: NM_001082597) was fused to the immunostimulatory epitopes P2-P30 (Ramirez-Andoney, V, et al. 2019) by adding a tail of 6 histidines at its 3' end, (1,260bp) and sent synthesize cloned in the pCINeo vector (Promega) obtaining the plasmid pCI-PAG10 (Biomatik, Toronto, CA). Likewise, the PAG10 6xHis gene was obtained synthetically cloned into the expression vector in insect cells pFastBac Dual obtaining the plasmid pFB-PAG10. The pCI-PAG10 plasmid was amplified and its DNA purified by EndoFree Plasmid Maxi Kit columns (Qiagen, CA, USA). The calculated DNA yield was 2.5 mg of DNA per 500 ml of bacterial culture with a 260/280 ratio of 1.70.

2) Production and purification of recombinant PAG10: The competent E. coli DH10Bac cells were transformed with the plasmid pFB-PAG10, this was purified to transfect Sf9 insect cells, using TransIT-Insect® Transfection Reagent (Mirus Bio LLC), obtaining the recombinant baculovirus FB-PAG10, which was used to infect SF9 cells (1x106/mL), at different multiplicities of infection (MOI). Recombinant PAG10 was purified by affinity chromatography with Ni+2-NTA nickel columns (Qiagen, USA). Protein identification was assessed by SDS PAGE electrophoresis and Western blot with an anti-His (H-15) antibody (Santa Cruz Biotechnology, CA, USA).

3) Obtaining monospecific polyclonal antibodies. Two groups of 4 male guinea pigs (50g each) and 2 groups of 2 male rabbits (500g each) were used. An experimental group of each species was immunized with an inoculum containing 100 µg of pCI-PAG10 plasmid DNA in a volume of 500 µl. The second experimental group was inoculated with 500 µl of PBS. Both groups were immunized on days 0, 14, 28 and 42; Animals were sacrificed on day 63 after immunization and serum was stored at −20°C until use.

4) To evaluate the reactivity of hyperimmune rabbit and guinea pig sera with the recombinant protein, sensitize a 96-well plate with serial double dilutions of recombinant PAG10 [1ug/ul] starting with 10ug of the purified recombinant protein and a constant dilution of the rabbit hyperimmune serum and whose 1/10 and 1/100

Results

At this time, the production of recombinant PAG10 in the baculovirus system has been obtained, as well as its purification. Likewise, rabbits and rabbits have been immunized with the gene vaccine and the evaluation of the specific antibodies produced, the purification of the IgGs and the standardization of the capture ELISA, as well as its validation, are pending.

Conclusions

This project seeks to develop a rapid, practical, economical, non-invasive and specific method for pregnancy diagnosis based on the detection of PAG in sheep, which allows strategic intervention in the reproductive management of the sheep flock. This project

proposes a technology for the production of monospecific polyclonal antibodies through genetic immunization and expression of recombinant proteins. The results obtained so far are the basis for carrying out the next step, which is the validation of the capture ELISA system. For this, a collection of serum samples and embryos from sheep with different gestation times have been obtained.

1562 - Use of pooled serum samples to determine flock-level prevalence of Anaplasma phagocytophilum antibodies in Irish sheep.

Author: John Gilmore, Seamus Fagan, Christine Hurley, Kate O Keefe, Amalia NaranjoLucena, Mark Hennessy, John F Mee, Tim Keady, Annetta Zintl

Objectives

Anaplasma phagocytophilum is an obligate intracellular Gram negative bacterium. The pathogen is transmitted by ticks, with *Ixodes Ricinus* being responsible for transmission of the pathogen in Ireland. *Anaplasma phagocytophilum* causes Tick Borne Fever, a disease of ruminants associated with immunosuppression, abortion, milk drop, fever and general malaise. Studies in other countries have shown sheep flock-level prevalences of antibodies to *Anaplasma phagocytophilum* of between 63 and 94%; no such studies have been carried out in Ireland. This study aimed to determine the seroprevalence of *Anaplasma phagocytophilum* in the Irish national sheep flock and to assess the use of pooled serum testing for this purpose.

Material and methods

Serum samples from 376 flocks were collected as part of a national *Brucella melitensis* surveillance study carried in 2019. As part of the study, 20 samples were taken from randomly selected sheep flocks throughout Ireland. Testing for the presence of *Anaplasma phagocytophilum* antibodies was done using a commercial inhibition ELISA kit (Anaplasma antibody Test Kit, cELISA, VMRD, Pullman, WA 99163 USA). Samples were initially tested using a pooled testing technique, with 5 samples from each flock being used to make a composite sample. This was followed by individual testing of each of the 20 samples collected from the flocks which showed a positive pooled result and a corresponding number of flocks with negative results on pooled testing

Results

In total, 36 flocks showed a positive result on pooled testing (9.6% flock prevalence). Hence, 36 positive and 36 negative flocks were tested individually. The greatest number of positive flocks originated from regions (counties) with a high predicted tick prevalence, with Cork South and Sligo/Leitrim/ Longford being the regions with the greatest numbers of positives.

The average percentage inhibition of pooled samples from positive flocks was 45.1, [95%CI 41, 49.3]. Pooled samples from negative flocks showed an average percentage inhibition of -15.1, [95% CI -17.2, -12.9]. The standard kit positive cut-off threshold is 30% inhibition.

Individual testing of 20 samples from positive flocks showed a mean % inhibition value of 22.1, [95% CI 19.9, 24.2]. Individual testing of samples from negative flocks showed a mean % inhibition value of 2.34, [95%CI 1.12, 3.56]. Within-flock seroprevalences of 40% and 5.8% were found in positive and 'negative' flocks, respectively.

Individual results were also interpreted using an adjusted cut-point of 19% (as suggested by other authors)(Scoles et al., 2008). Using this adjusted cut-point, every positive flock (on pooled testing) had a minimum within-flock prevalence (on individual samples) of 15%. Using the same 19% cut-point in negative flocks (on pooled testing) 9 flocks previously identified as negative using pooled tests are reclassified as positive with a 15% or greater prevalence

The sensitivity and specificity of pooled sample analysis was investigated by calculating receiver operating characteristics (ROC), (Sergeant, 2018) . ROC calculations indicated that a cutpoint of 2.048% had a sensitivity of 91.1%, [95% CI 79.3,96.5] and specificity of 92.3%, [95%CI 75,9. 97.9], when used to detect infected flocks. Here an infected flock wa defined as one with 15% or greater within-flock prevalence, based on an individual animal sample cut point of 19% inhibition or greater.

Conclusions

A lower cut-point than that recommended by the kit manufacturer should be used when analysing pooled serum samples from sheep flocks to establish the flock-level presence of infection to *Anaplasma phagocytophilum*. The use of a pooled samples made up of 5 individual samples and a percentage inhibition cut point of 2% allows a simple, cost-effective means of screening flocks for the presence of *Anaplasma phagocytophilum* antibodies. In this case infected flocks were regarded as those with 15% or more positive animals when measured individually using the same kit but where an adapted cut off of 19% inhibition was used.

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1583 - Melatonin Administration in Late Pregnancy : Effects on Colostrum Quality and Lamb Growth in Merino Ewes**

Author: Şule Bircan, hakan üstüner

Objectives
Colostrum is a vital source of nutrients and immunoglobulins for the neonate, and an adequate supply of colostrum significantly increases the chance of survival to weaning. Effect of late pregnancy melatonin administration to Merino ewes on colostrum quality and lamb growth performance.

Material and methods
The research was carried out at Bursa Uludag University Faculty of Veterinary Research Center (40.2350° North latitude, 28.9250° East longitude). The sheep to be used in the research were followed up starting from the breeding season. Pregnant ewes were identified by ultrasound on the 30th day after insemination. Pregnant sheep were divided into 2 groups. We take into account age and single or twice lamb. 18 mg of melatonin was applied as an ear implant to the animals in the experimental group (n=11). No administration was made to the control group but for the placebo effect, a 1 cc isotonic solution was administered to the control group from the same part of body (n=10). Melatonin and placebo were performed in the last 45 days of pregnancy. The birth period was followed closely. The colostrum quality of the giving birth was measured in the first 30 minutes. Colostrum quality was evaluated by refractometry. The birth weight of lambs was weighed within half an hour after birth. The lambs were weighed every 20 days until day 120. Evaluation of the data was made with SPSS statistical program, GLM and Student-t test.

Results
Colostrum quality was 36,52± 5,56 and 30,96± 9,69 in the experimental group and control group, respectively (P<0.05). Lamb birth weights were 3,47±0,87 kg in the experimental group and 3,51±0,86 kg in the control group (P>0.05). The weights of the experimental grup's lambs at 60, 90 and 120 days of age were 17,72±3,71, 31,50±7,85 and 36,14±9,12 kg, respectively. The weights of the control grup's lambs at 60, 90 and 120 days of age were16,88 ± 4,26, 30,31±11,30 and 35,04±12,96 kg, respectively. The differences between the experimental and control groups in terms of weights were not statistically significant.

Conclusions
The main finding of the study was that colostrum brix was high in the experimental group. It can be recommended for use in ewes with low colostrum quality. No differences were found in the weight at birth and in the growth performance (P>0.05). Suggests that further research with larger numbers of animals would provide a clearer understanding.

1602 - Use of glutamic acid to improve the motility of cooled semen from Dorper sheep

Author: Laura Gabriela Ibarra Compian, Ma. Guadalupe Calderon leyva

Objectives

Evaluate the effect of glutamic acid on the motility of chilled sheep semen.

Material and methods

The study was carried out at the Universidad Autonoma Agraria Antonio Narro Unidad Laguna in Torreón Coahuila México with the coordinates 25.556811 north, -103.372450 west. Two pubescent rams of the Dorper breed were used, with an age of 10 months and a body condition of 3.5, reproductively healthy, with prior adrological evaluation by ultrasonography. They were extracted through the use of an artificial vagina as described by Carrera *et al* in 2020.In a period two months, twice a week. Once the semen was obtained, it was tempered at 37 °C in a water bath for the evaluation of concentration using a photometer, as well as the evaluation of mass motility, motility and viability using eosin nigrosin staining.Once the samples were tempered, the commercial diluent "optixcell 2" was added. Leaving the sample at a concentration of 200 million, and then dividing the sample into two groups, one group added 20 microliters of glutamic acid at a concentration of 0.08. mg per milliliter, leaving the other sample as a control group. The two samples were refrigerated for four hours to be analyzed in the CASA system. These results were analyzed in analysis of variance using the General Linear Model (PROC GLM) procedure.While to determine the effect of the treatment, it was evaluated by comparing the means using the Duncan test, using the SAS 9.0 statistical package (Inst. Cary, NC, United States of America).

Results

The results are presented with their respective standard error. They were obtained after the semen refrigeration period and do not present a significant difference ($p>0.05$) in any of the variables.Total motility, the control group obtained 73.01% ± 1.76 and in the glutamate group 74.88% ± 1.20, progressive motility, the control group obtained 71.93$ ± 1.76 and in the glutamate 74.44 ± 1.23, rapid progressive motility,the control group of 56.13% ±2.05 and in the glutamate group 56.42 ± 1.81, slow progressive motility, the control group obtained 15.69% ±1.15 and the glutamate group 17.55% ± 1.25 local motility, in the control group its results were 0.53% ± 0.06 and in the glutamate group 0.52 ± 0.04 and immobile, in the control group 26.63% ± 1.62 and in the glutamate group 25.02 ± 1.19 affirming the null hypothesis of the treatment, therefore the acid Glutamic acid at a concentration of 0.08 milligrams has no effect on the motility of chilled sheep semen.

In the study carried out by Alvarez et al in 2019, glutamic acid has an effect on sexual behavior, puberty and penile erection in mammals, but it has no relationship with the process of spermatogenesis, seminal quality or sperm motility. In addition to the fact that Jubaidi et al in 2019, after exposing male mammals to doses of exogenous glutaman, expressed deteriorating effects on semen characteristics; low semen concentration, as well as low sperm motility and viability, but not significantly different from the control group, as in the current study. Sperm have a high number of glutamate receptors, causing damage by extraexcitation of the cell thanks to the addition of exogenous glutamate

Conclusions

The addition of glutamate to refrigerated ram semen does not present a statistical difference between the groups in any of the motilities presented by the CASA system, and even an excessive amount of the acid is harmful to the semen and its viability as presented in the literature, proposing that its use does not benefit the sperm or its motility.

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1606 - FENBENDAZOLE RESISTANCE OF NEMATODES GASTROINTESTINAL IN SHEEP AT THE RANCH "LOS LIMONES" FROM PITAYO, GUERRERO, MEXICO

Author: Mario Antonio Mendoza Nuñez, Baltazar Timoteo Cruz Chavez, Jorge Axel Moran Nava, Citlali Estefania Sollano Mendieta, José Fernando Olvera Rámirez, Luis Antonio Saavedra Jiménez

Objectives

Determine the effectiveness of fenbendazole (FZ) against gastrointestinal nematodes through the egg count reduction test in feces.

Material and methods

The present study was carried out in the Los Limones production unit, located in Pitayo, San Nicolás, Guerrero, Mexico. Two groups of 43 females each were formed, with nematode loads greater than 200 eggs per gram of feces (H.P.G). With an average load of 3263 H.P.G for the control group and 3487 H.P.G for the treatment group. Oral Fenbendazole was applied to the treatment group at 5 mg/kg body weight. The parasite load was determined using the MC-Master method (Sixtos et al., 2011) and the data were used to determine the percentage of load reduction and the presence of anthelmintic resistance according to the methodology proposed by Coles et al., (1992).

Results

The control group showed an increase in the average parasite load of 4.5%, while the treated group showed a decrease in the load of 31%, demonstrating that FZ had an effect on the NGI, however, through the reduction test egg count, it was determined that the percentage reduction of the NGI of the treated group compared to the control group was 68% (Lim-Cinf: 31 and Lim-Csup: 86), which indicates the presence of resistance to FZ to dose of 5 mg 25 kg-1 live weight (Table 1), according to the criteria proposed by Coles (1992). Resistance to this anthelmintic has been previously reported in other parts of the country, in Mexico, Montalvo et al. (2006) reported a reduction of 87%. While Cuellar and Soberanis (2013) reported a reduction of 84%. Reyna et al. (2023) in Tamaulipas report a reduction of 18% towards the FZ. However, it is important to highlight that this study is the first report in the Costa Chica of Guerrero.

Conclusions

In the animal production unit “Los Limones” the prevalence of nematodes (strongylids and Strongyloides) was 74%, with an average parasite load of 3335.6 hpg.

Gastrointestinal nematodes of sheep in the animal production unit “Los Limones” showed resistance to Fenbendazole, through the reduction test in egg counting.

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1612 - Epidemiological and clinical study of Anaplasma ovis in sheep herds in Brazil

Author: Gisela Gregoria Choque , Lucas Alencar Fernandes Beserra, Marian Angeles Ramo Gil, Marcia Mayumi Fusuma, Líria Hiromi Okuda, Lilian Gregory

Objectives
The objective of this study was to evaluate the occurrence of animals affected with clinical manifestations suggestive of *Anaplasma ovis* in sheep herds in Brazil.
Material and methods
A total of 104 blood samples were collected from sheep from herds in the municipality of Buri-SP, Vinhedo-SP, Serra Negra-SP, Itu-SP, Recife-PE, 81.7% (85/104) females and 18.3% (19/104) males, of the Texel, White Dorper, Dorper, Poll Dorset and Santa Inês breeds. Hematimetric and serum biochemical parameters were evaluated, with a questionnaire with closed questions and a physical examination form being applied to each property with the purpose of obtaining information focused on animal management and health parameters, respectively. For molecular diagnosis, the real-time PCR technique was used and they were processed in the Laboratório de Viroses de Bovídeos, at the Instituto Biológico de São Paulo, São Paulo-SP. DNA was extracted using the commercial MagMAX CORETM Nucleic Acid Purification Kit (Thermo Fisher Scientific Inc., Waltham, MA, United States) in an automated DNA/RNA extraction system (KingFisherTM Flex Purification System). For amplification, the commercial EXOone Anaplasma spp. kit was used. oneMIX qPCR (EXOPOL SL, Zaragoza, Spain) and the EXOone Anaplasma ovis oneMIX qPCR kit (EXOPOL SL, Zaragoza, Spain), following the manufacturer's recommendations, being carried out on a QuantStudioTM 12K Flex equipment (Applied Biosystems) and the results were analyzed with the respective software (software v1.5)
Results
Samples for molecular diagnosis were negative for *Anaplasma spp* and *A. ovis*.
Conclusions
In the study, it was not possible to collect samples from all States due to the project's short research time, but the presence of this bacteria varies greatly across continents, depending mainly on the geographic region and the presence of vector ticks. Given this, more long-term research should be applied to detect and analyze the complexity of the different anaplasma genotypes existing in Brazil. Such as implementing control measures and sanitary management of flocks, thus generating quality, productivity, health and well-being in Brazilian sheep farming.

1615 - NONLINEAR MODELS TO DESCRIBE THE GROWTH OF COLUMBIA SHEEP IN MEXICO

Author: Ricardo Emmanuel Martinez Rocha, César Garzón Pérez, Victor Díaz Sánchez

Objectives
The study's objective was to determine the best nonlinear model to fit the growth curve of Columbia sheep in the central zone of Mexico.
Material and methods
The study was carried out in the locality of Cuautitlan, State of Mexico. A total of 174 Columbia Sheep (92 males and 84 females) were used for the experiment. Live weight records were recorded during several times of their lives, a total of 1,887 records were obtained. The records were considered from birth to 450 days of life. Only sheep with more than three weight records were considered in the study. The sheep considered were fed two times per day with corn silage and oat hay. Animals were weaned with 75 days of life on average. The nonlinear models to characterize the growth curve in this production system were the Brody, Logistic, Von Bertalanffy, and Gompertz models. In the four models, the parameters of mature weight in kilograms (A), integration constant (b), and the maturity rate of the animal (k) were considered. Each parameter was considered a fixed effect. The models were evaluated for each sex. The Akaike Information Criterion was used to define which growth model is appropriate.
Results
The best model for characterizing the growth curve in Columbia sheep was the Brody model for females and the Gompertz model for males. The parameters estimated with this model for females were A=39.7 kg, b=0.86, and k=0.006; for the males, they were A=43.8 kg, b=2.11, and k=0.013. These parameters can be useful in this breed under the same conditions of production.
Conclusions
The Brody and Gompertz nonlinear models were the most appropriate for characterizing the growth curve in Columbia sheep with the production system mentioned for females and males respectively.

1635 - ULTRASOUND EVALUATION OF THE URETHRA OF NON-CASTRATED TEXEL LAMBS

Author: Priscilla Fajardo Pereira Valente, Marina Franco, Emelly Selmer Moura, Mayara Cardoso Anjos, Lisandra Camargo Campos, Nicoly Nayana Marcom, Júlio Augusto Naylor Lisboa

Objectives

Obstructive urolithiasis is an important disease in sheep farming, which is a common condition of intensively reared rams, and is associated with early culling of animals, treatment costs and death of affected animals. Ultrasonographic examination can provides useful information in male sheep with suspected partial or complete urethral obstruction. The objective of this study was to evaluate the efficacy of ultrasonography with two differents probes (external linear and transretal linear) in the examination of the penile urethra of non-castrated Texel lambs in the growth phase, intensively raised.

Material and methods

Twenty-five healthy Texel lambs were used and subjected to weighing and physical examination at 2, 4 and 5 months of age, before each ultrasound evaluation. The ultrasound examination took place with the animal under physical restraint, in the right lateral decubitus position. Trichotomy of the areas defined for taking images was done with a portable hair trimmer. Ultrasound conductive gel was applied between the skin and the transducer to increase the contact surface and ensure the best quality image possible. The urethra was evaluated at four specific locations: a) proximal urethra (perineal region); b) sigmoid flexure (caudal aspect of the base of the scrotum); c) cranial to sigmoid flexure (cranial aspect of the base of the scrotum); d) distal urethra (1/2 of the distance between the glans penis and the base of the scrotum). The images were taken in cross sections. At each location, diameter measurements were taken when the urethra had a circular shape. The ultrasound device used was the Esaote Veterinary model MyLabDelta. Measurements were carried out in B mode using two types of transducers: linear transducer for external use (SL 1543) and transrectal linear transducer (SV 3513), setting the highest possible frequency for each (13 MHz and 10 MHz, respectively). Two-factor repeated measures analysis of variance was used to test the effect of age (2 × 4 × 5 months), the effect of linear transducer type (external × transrectal use), and the interaction between the two factors. The Tukey test was used to compare means, assuming a probability of error of 5%.

Results

In all anatomical regions analyzed, it was possible to observe the urethra. The urethra diameters found at different ages were statistically different, demonstrating an increase in the diameter of the urethra as the lamb grows. Using the external linear probe, the following means were obtained: 1.79 mm at two months and 2.27 mm at five months of age in the perineal region (site a); 1.30 mm at two months of age and 2.15 mm at five months of age in the sigmoid flexure (site b); 1.35 mm at two months of age and 2.00 mm at five months of age in the pretesticular region (site c); 1.47 mm at two months of age and 1.94 mm at five months of age in the penile region (site d). Using the transrectal linear probe, the following means were obtained: 1.62 mm at two months and 2.52 mm at five months of age in the site a; 1.40 mm at two months of age and 2.25 mm at five months of age in the site b; 1.45 mm at two months of age and 2.30 mm at five months

of age in the site c; 1.61 mm at two months of age and 2.17 mm at five months of age in the site d. There was a statistical difference between the probes in the assessments at 4 and 5 months of age in locations c and d, among them, the transrectal linear presented higher averages.

Conclusions

It was possible to observe an increase in the diameter of the urethra in all anatomical locations designated for the examination, as the lambs grew. The external linear and transrectal linear probes are valid instruments for the ultrasound examination of the urethra and can help in the early diagnosis of suspected cases of obstructive urolithiasis in the field.

1642 - Ultrasound characteristics of the spleen and liver in healthy hair sheep

Author: Tatiane Vitor da Silva, Bianca Paola Santarosa , Rafael do Prado Freitas, Beatriz Souza Costa, Claudilene Rodrigues Carvalho, Bruna Ferreira Valle, Jobson Filipe de Paula Cajueiro, Mário Felipe Alvarez Balaro

Objectives

Ultrasonography (US) in sheep has been widely used in research and clinical routine, because it is a non-invasive and practical exam used for diagnosis of several diseases. However, there is little information about the liver and spleen of healthy ovine to better understand when these organs have alterations. Therefore, it was aimed to describe qualitative ultrasound attributes of the spleen and liver in healthy hair ewes.

Material and methods

This study was approved by an ethical committee for animal use (n. 2919170821). To attest to animals' health, a thorough examination was carried out encompassing clinical assessments and laboratory analyses, such as complete blood count and liver biochemistry. Following this, transabdominal ultrasonography was applied to scrutinize the spleen and liver of 35 Santa Inês adult ewes (average age of 4.9 ± 0.7 years; weight of 49.2 ± 8.7 kg; and a body condition score of 3.0 ± 0.1), which were being kept in an intensive system. The ultrasound assessments were conducted using portable B-mode equipment fitted with 7.5 and 5.0 MHz linear and convex transducers, respectively. Throughout all US scans, animals were maintained in standing positions contained by a trained person. A general ultrasound examination of the spleen and liver were carried out in each intercostal space (ICS) with the transducer held parallel to ribs, beginning from dorsal-ventral and caudo-cranial directions. Echogenicity and echotexture the splenic and hepatic parenchyma, distance between the dorsal and ventral margins of both spleen and liver in relation to the midline of the back were measured along with the diameter of the splenic vein, caudal vena cava, portal vein, location, and size of the gallbladder (GB).

Results

All blood count and liver biochemistry levels of ewes were within normal ranges (aspartate aminotransferase - AST: 113.0±23.6 IU/L; alkaline phosphatase - ALP: 167.9±64.0 IU/L; gamma glutamyl-transferase - GGT: 47.6±11.5 IU/L; Total protein:7.0±0.7 g/dL; Albumin: 3.1±0.3 g/dL; Globulin: 4.0±0.5 g/dL). The spleen was visualized from the caudal region to the last rib, up to the 8th ICS, surrounded by a thin, smooth, hyperechogenic capsule. The splenic vein was seen as an anechoic structure with an oval to circular or elongated shape, and was located from the 12th to the 9th ICS, being seen in most animals between the 11th and 10th ICS and its diameter ranged from 1.2 to 3 cm. The liver was located from the 12th to the 7th ICS and circumscribed by a thin and smooth hyperechoic line. The portal vein had a maximum diameter of 1.9 cm a minimum of 1.0 cm, and a mean area of 187.2 cm, with a rounded shape and walls with an echogenic edge, especially in the vessels of greater amplitude. The caudal vena cava was seen in a triangular, elliptical, or round shape, and with less echogenic edges, with a diameter of 1.2 cm. The GB was seen with different shapes, alternating between

circular, oval, and pear-shaped, with anechoic to hypoechoic content and hyperechoic walls. Length ranged from 5.9 to 1.1 cm, measured area from 728 to 23 mm, and height from 19.7 to 2.8 mm. Compared with the liver, in the same animal, the spleen was sonographically hyperechoic in 11 sheep, isoechoic in 16, hypoechoic in three, and five, both organs were hyperechoic.

Conclusions

The current data showed that the US was a good tool to determine reference values for the spleen and liver of healthy hair sheep. Through the results obtained so far, it was possible to establish sonographic normality for these organs in sheep and it can be helpful for the clinical routine.

1647 - Ultrasonographic renal characteristics in healthy hair sheep

Author: Tatiane Vitor da Silva, Bianca Paola Santarosa , Rafael do Prado Freitas, Beatriz Souza Costa, Claudilene Rodrigues Carvalho, Bruna Ferreira Valle, Jobson Filipe de Paula Cajueiro, Mário Felipe Alvarez Balaro

Objectives

In sheep, ultrasonography (US) has been widely used in the evaluation of urinary tract lesions. However, little is known about the characteristics of renal structures. Therefore, it was aimed to describe qualitative ultrasound attributes of kidneys in healthy hair ewes.

Material and methods

This study was approved by an ethical committee for animal use (n. 2919170821). To evaluate the health of the animals, a comprehensive clinical examination and laboratory tests, encompassing a complete blood count and renal biochemistry were conducted. Subsequently, transabdominal ultrasonography was employed to assess the kidneys of 35 Santa Inês adult ewes, aged 4.9 ± 0.7 years, weighing 49.2 ± 8.7 kg, and with a body condition score of 3.0 ± 0.1, all maintained under the intensive system. The ultrasound assessments utilized portable B-mode equipment equipped with 7.5 and 5.0 MHz linear and convex transducers. Throughout all US scans, animals were maintained in standing positions contained by a trained person. For precise kidney assessment and localization, the right flank was divided into imaginary quadrants (Q2 and Q1), and the area of the 12th and 11th intercostal spaces (ICS) was demarcated. The right kidney (RK) was examined in the last two ICSs and the area immediately caudal to the last rib, while the left kidney (LK) was assessed in the imaginary Q2. Both kidneys were examined in longitudinal and cross sections, measuring length, height, and thickness based on longitudinal images. The three largest visible medullary pyramids' diameter, along with various other parameters such as corticomedullary and cortical diameter, pelvis diameter, ureter and renal hilum, and medullary diameter, were determined from transverse images. Subjective evaluations included the echogenicity and echotexture of the cortical and medullary regions.

Results

The hematological and renal biochemical parameters of ewes remained within the reference range for the species (urea: 20.0±8.4 mg/dL; creatinine: 0.8±0.3 mg/dL). The RK was seen in the craniodorsal quadrant (Q1) and at the 12th ICS juxtaposed to the caudal pole of the liver (caudate lobe), and the LK only in the right caudodorsal quadrant (Q2). The ultrasonographic appearance of the kidneys changed according to the sectional plane. In the longitudinal section, the kidney was seen in an elongated oval shape with homogeneous parenchyma and a thin hyperechoic capsule that surrounded it. The renal cortex appeared hypoechoic and several medullary pyramids were identified and appeared as round to oval, hypoechoic to anechoic structures. In the cross-section, the kidney presented a rounded to oval shape, circumscribed by an echogenic line, the renal hilum was seen as a hyperechogenic band from which the renal vein and artery and the ureter came out, which appeared as a thin hyperechogenic line without a visible lumen. The length (RK: 7.6 to 5.1 cm; LK: 7.5 to 5.1 cm), the height (RK: 4.5 to 3 cm; LK: 4.8 to 2.8 cm), and the thickness (RK: 4.7 to 3 cm; LK: 4.7 to 3.2 cm) were

measured for both kidneys. The mean capsule thickness was 0.14 cm for RK and 0.13 cm for LK. The diameter of the three largest medullary pyramids in the RK ranged from 0.5 to 1.4 cm; in the LK, the values obtained ranged from 0.5 to 1.2 cm. The corticomedullary, cortical, and medullary diameters were assessed (RK: 1.3 cm, 0.75 cm, and 0.6 cm; LK: 1.3 cm, 0.73, and 0.56 cm, respectively). The diameter of the ureter (RK: 0.19 to 0.1 cm; LK: 0.18 to 0.1 cm), the diameter of the renal pelvis (RK: 0.3 to 0.1 cm; LK: 0.28 to 0.1 cm), and the diameter of the hilum (RK: 1.0 to 0.4 cm, LK: 0.88 to 0.43 cm) were also evaluated.

Conclusions

This study indicated that the US was a valuable tool in establishing reference values for the kidneys of healthy Santa Inês adult sheep. Based on the findings thus far, it has become feasible to define sonographic norms for renal characteristics in sheep, offering potential assistance in clinical practice.

1654 - Colostrum quality on intensive Greek dairy sheep farms

Author: Sofia- Afroditi Termatzidou, Panagiota Kazana, Juliana Mergh Leão , Leonidas Rompies, Manuel Campos

Objectives
The objective of the study was to obtain information regarding colostrum quality of intensively reared Lacaune ewes in Greece, as determined by Brix refractometry.
Material and methods
The study was conducted in 9 commercial Lacaune sheep flocks in Central and Western Greece, from October to December 2023. Flock size ranged from 270 to 350 milking ewes and a minimum of 10% of the ewes from each flock were sampled once during the test lambing period. Inclusion criteria for the selected ewes were; clinically healthy, age (2-4 years), body condition score (≥ 2.75), dry period length (> 60±12 days), vaccination for enterotoxemia 20-30 days prior to lambing date. Similar nutritional management practices were applied during the dry period and all ewes were offered the same concentrate supplement. In total, first milking colostrum was obtained from 245 ewes by hand milking and stored at -20°C until analysis. Sheep id, collection date, time interval between lambing and milking, prolificacy, gender of lambs and dystocia were recorded. Total solids (TS) concentration of thawed colostrum samples was measured using a digital Brix refractometer (Palm Abbe PA203x, Misco, USA). All measurements were performed in duplicate and the average was recorded. Measurements of < 22% were considered indicative of poor colostrum quality, 22-26% as fair quality and > 26% as good quality. Data were analyzed using IBM SPSS v.22.0 (Armonk, NY, USA: IBM Corp.). Time interval between lambing and colostrum collection was grouped as ≤2, 3-6, 7-12 and 13-24 h. Linear regression with TS as the dependent variable was used to examine the associations between colostrum quality and the different independent variables. Significance level was set at $p < 0.05$.
Results
The mean Brix value (±SD) was 21.75±4.62% and ranged from 11.10% to 34.20%. Colostrum was classified as poor in quality in 55.51% of the samples, while 25.71% of the farmers collected and provided colostrum to their lambs > 7h after lambing. Time interval between lambing and milking, farm and prolificacy had a significant effect on TS ($p<0.005$). The gender of each lamb was considered in the initial screening but no association ($p=0.216$) was observed with TS and, therefore, were not entered in the final model. Estimated marginal means with 95% confidence intervals produced, for time interval between lambing and milking are 23.77 (22.66-24.87), 20.67 (19.61-21.73), 19.98 (18.46-21.51) and 17.58 (15.42-19.74) for ≤2, 3-6, 7-12 and 13-24 h respectively.
Conclusions
More than half of the collected samples were classified as poor in quality and further investigation is needed to assess factors and management practices affecting colostrum TS in intensive dairy sheep flocks in Greece. As it was expected, time interval between lambing and milking and farm affected significantly colostrum quality. Greek farmers should be motivated to implement colostrum management protocols in their routine during lambing periods.

1665 - IMAGE DIAGNOSIS IN THE HEALTH MANAGEMENT OF RETROVIRAL PROCESSES IN SMALL RUMINANT FARMS

Author: João Jacob-Ferreira, Ana Cláudia Coelho, Delia Lacasta, Ramiro Valentim, Hélder Quintas

Objectives

Ovine pulmonary adenocarcinoma (OPA) and Small ruminant lentivirus (SRLV) are both retroviral diseases that affect small ruminants. They are associated to high economic losses in livestock farms owing to the fact these infections are debilitating, progressive and longstanding. Calling upon to diagnostic imaging techniques to characterize lesions developed by retroviral infection in small ruminants was the main aim of this work.

Material and methods

This study was carried out at the Pedagogical Animal Husbandry Unit of the Agrarian School of Bragança (Universities of Applied Sciences). With clinical suspicion and when suspecting of ovine pulmonary adenocarcinoma (OPA) or lentivirus infection (SRLV) animals were submitted to complementary diagnostic tools of imaging. Diagnosis was confirmed through necropsy, histopathology and PCR or microbiology.

Results

Lung lesions compatible with OPA presented, on ultrasound, echogenic areas of different sizes corresponding to neoplastic nodules. Chest x-ray shown nodules of different sizes and locations and sometimes a nodular pattern with diffuse nodules. CT (Computerized Tomography) accurately detected tumour nodules, their size and location.

In ultrasound examination (US) when exploring different regions of the lung it aimed to understand the distribution of lesions. An evident increase and homogeneous in echogenicity from a consolidated parenchyma was due to chronic interstitial pneumonia. While a high and homogeneous echogenicity in mammary parenchyma was found in chronic indurative mastitis. Radiography (X-ray) and computed tomography scan (CT) were also used in this study, despite the limitations associated with health, safety regulations, associated costs and the use of ionizing radiation. These tools were useful to understand the pathological processes of SRLV infection, mainly at respiratory level. In more advanced stages, X-ray have shown a widely distributed unstructured diffused interstitial pattern, with bronchogram sign in lungs. Increased opacity in lungs can be due to infiltration of lymphocytes in the interstitial tissue. Thoracic-CT provides a better detail of the lesion scan which enable visualizing a uniform increment of radiopacity in several planes minutely.

Conclusions

Using different imaging exams this work aims to describe lesions developed by retroviral diseases in small ruminants. This way, we intend to support the veterinarian in identify and characterize these lesions. These tools can be used as a complement to accurate clinical and laboratory examination, avoiding underdiagnosis and contributing to the implementation of appropriate disease control measures. Some techniques (X-ray and CT) will not be able to be used at the field level but are useful for academic purposes. Ultrasound is, in its turn, a non-invasive modality already used on farms for reproductive diagnosis. Therefore, this resource, with the increasing availability of portable digital ultrasounds, can allow professionals to quickly and immediately evaluate animals on a

farm level, potentially making this diagnostic tool more accessible and thus playing an important role in daily clinical practice.

Acknowledments and funding:
Projeto: 0687_OVISPID_2_E POCTEP – Programa de Cooperação Transfronteiriço Portugal – Espanha
This study was also supported by projects UIDB/00772/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

1667 - RISK FACTORS ASSOCIATED WITH LIVER CONDEMNATION AT THE SLAUGHTERHOUSE IN LAMBS CHURRA DA TERRA QUENTE BREED

Author: João Jacob-Ferreira, Jorge Azevedo, Hélder Quintas, Madalena Vieira-Pinto

Objectives
The *Churra da Terra Quente* (CTQ) sheep are an indigenous Portuguese breed, produced in a semi-extensive and traditional way. In lamb production, risk factors may occur that may favour the development of diseases or injuries which may constitute causes of rejection in slaughterhouse. This work aimed to study possible risk factors on farm associated with the occurrence of liver rejection in the CTQ lambs during *post-mortem* inspection in a slaughterhouse.
Material and methods
The study was carried out at the slaughterhouse, where meat inspection of the CTQ lambs breed was monitored and causes of liver condemnation were recorded. Subsequently, a survey was performed at the farm level to identify possible risk factors.
Results
In this study, 35.2% (n=166/472) lamb livers were rejected. The main cause of liver rejection was essentially lesions compatible with parasitism (98.8%). The rejection rate due to parasitism was significantly higher ($p<0.05$) in lambs from farms whose owner had no professional training and in which there was no individual food store, and highly significant ($p<0.0001$) in farms where guard dogs were not dewormed, and pastures were shared with other herds.
Conclusions
We concluded that risk factors in the production of the CTQ sheep may favour the development of diseases and lesions in lambs' livers, which could possibly be rejected during health inspections in slaughterhouses. Greater and better knowledge about risk factors that can influence animal health on farms should promote an opportunity in risky behaviour change in production, with the aim of mitigating negative effects on animal health and welfare, as well as improving the profitability of farms. It should be added that the identification of risk factors on farms and their communication to the Official Veterinarian may contribute to improve the effectiveness of health inspection. Similarly, it is useful for the producer that the veterinary doctor to inform about meat inspection results. Creating an active and bidirectional communication between the producer and the Official Veterinarian is important to improve the health profile at herds and for the performance of the medical-veterinary activity at farm and slaughterhouse level.

Acknowledments and funding:
Projeto: 0687_OVISPID_2_E POCTEP – Programa de Cooperação Transfronteiriço Portugal – Espanha
This study was also supported by projects UIDB/00772/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

1682 - Culture and antibiogram of mandibular abscesses in sheep in the state of Tolima, Colombia

Author: Helio Martins de Aquino Neto, Irma Ximena Barbosa Sanchez, Jose Alexander Correa Díaz

Objectives

The identification of the presence of mandibular abscesses in small ruminants, often associated with local osteomyelitis, has been increasingly reported in the global scientific literature. In cattle, many cases are associated with *Actinomyces bovis*, a disease known as lumpy jaw. In goats and sheep, the etiology of these mandibular lesions is highly varied and can also be caused by infections from *Actinobacillus lignieresii*, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, and *Arcanobacterium pyogenes*. The economic impact on production is considered significant, especially when the roots of molars are involved, leading to pain during feeding and often accompanied by progressive weight loss in the animal. The main cause of the problem is believed to be oral cavity traumas that create conditions for infection by opportunistic pathogens. Etiological diagnosis of this clinical condition is essential to propose more efficient treatments and prevention methods. The aim of this work is to report three cases of mandibular abscesses in sheep, accompanied by culture and antibiogram analysis.

Material and methods

Cases are reported involving three crossbred sheep from different properties managed in an extensive production system, with a diet based on pasture and mineral salt. To facilitate the presentation of the results, the animals were identified by numbers 1 (origin: Armero), 2 (origin: Natagaima), and 3 (origin: Ibagué), all from municipalities located in the state of Tolima, Colombia. The animals shared, in addition to low body condition, an increase in volume in the mandible, firm at the edges and softer in the center, with pain on palpation and approximately 3 to 5 cm in diameter. For material collection, trichotomy was performed, followed by disinfection to make an incision and drain the pus. Collection was done using a sterile swab, transported to the laboratory in thioglycolate broth for etiological diagnosis. The sample was seeded on blood agar and McConkey agar and incubated at 37°C for 24 hours for culture and antibiogram analysis.

Results

In the microbiological culture of the collected samples, there was bacterial growth of *Streptococcus* spp. in animal 1, *Pseudomonas aeruginosa* and *Corynebacterium pseudotuberculosis* in animal 2, and *P. aeruginosa* in animal 3. Regarding the antibiogram, the sample from animal 1 showed multidrug resistance (penicillin G, tetracycline, trimethoprim/sulfamethoxazole, erythromycin, ampicillin/sulbactam), with intermediate sensitivity to ciprofloxacin. For animal number 3, the results were the opposite, where the isolated agent showed sensitivity to almost all tested antibiotics (ciprofloxacin, norfloxacin, gentamicin, amikacin, and cefepime), showing resistance only to cefotaxime. The marked difference in results found here is in accordance with the various treatments proposed in the literature, where there is no unanimity among the consulted authors, and a wide variety of antibiotics is used, often with very different

and not always satisfactory results. Long-term follow-up of the evaluated animals was not possible, and no type of treatment could be established because animals 1 and 3 were sold by the owners' decision, as they were commercial herds. Animal 2 died three days after its clinical evaluation due to a snakebite accident. The findings of this report confirm the diversity of etiological agents that may be involved in cases of mandibular abscesses in sheep. In many cases, the prognosis is reserved due to possible osteomyelitis with dental involvement, leading to high treatment costs, reserved prognosis, and slow recovery when it occurs.

Conclusions

Pseudomonas aeruginosa, *Corynebacterium pseudotuberculosis*, and *Streptococcus* spp. are involved in the etiology of mandibular abscesses in sheep. Due to the currently known predisposing factors for the problem, which are mouth injuries, it is crucial for reducing its economic impact on productive systems to adopt preventive measures that minimize the risks of oral cavity injuries. This can be achieved primarily through the provision of higher-quality food, as well as proper training of employees to use syringes and oral dosing guns correctly.These devices can serve as entry points for opportunistic bacteria. Cultural and antibiogram studies of material collected from mandibular abscesses are essential for establishing a more standardized treatment for the problem in the future. This approach offers better possibilities for the recovery of affected animals.

1765 - Clinical outbreak in sheep flock caused by Theileria ovis and Anaplasma spp. in southeastern Brazil

Author: Nathalie Costa da Cunha, Matheus Dias Cordeiro, Isadora de Fatima Braga Magalhaes, Julia Cardoso Pereira, Bruna de Azevedo Baêta, Mário Felipe Alvarez Balaro

Objectives
Hemoparasites are distributed worldwide with great significance in veterinary medicine. However, there are few reports involving hemoparasites in sheep from Brazil, mainly because there are no ticks as primary host in small ruminants, with parasitism being accidental. This study aimed to report the detection of *Theileria ovis* and *Anaplasma* spp. in ovine species, besides to clinical and hematological features in a meat sheep flock from southeastern Brazil.
Material and methods
Animals from a mob of 16 adults Santa Ines ewes, newly acquired by the farmer, showed broad clinical signs of nasal discharge, conjunctivitis, lung crackles, cough, enlarged lymph nodes, pasty stools, fever, thinness and poor appetite. Moreover, ticks inside the ear and inner thigh in some animals were found. As complementary tests, fecal egg count and blood samples for whole blood count and molecular exams were collected. Clinical ultrasonography was also performed on three most affected animals. All blood samples were subjected to polymerase chain reaction assay after DNA extraction. The DNA samples were screened for a partial 18S rRNA gene fragment from the Piroplasmida order, a partial 16S rRNA from *Anaplasma* spp., dsb gene for *Ehrlichia* spp. Electrophoresis in agarose gel was performed on the amplification products, and the ethidium-bromide-stained gels were visualized under ultraviolet light. All PCR-positive samples were submitted to Sanger sequencing.
Results
Among all animals (n=16), 37.5% (6/16) were PCR-positive for Piroplasmida order, 12.5% (2/16) exhibited positive PCR results for *Anaplasma* spp. and no animals were positive for *Ehrlichia* spp. A total of 66.7% (4/6) of the piroplasm-positive animals were infested with *Rhipicephalus* (*Boophilus*) *microplus* ticks, and 66.7% (4/6) presented clinical signs. Furthermore, the hematological evaluation from the six piroplasms-positive animals revealed the following mean values: White blood cells (WBC) (15.9±2.2 $x10^3$/µL; leukocytosis), hematocrit (Hct) (26.0±1.2%), red blood cell (RBC) (8.4±0.5 $x10^6$/µL; anemia) and platelets (PLT) (706.0±58.0 $x10^3$/µL; thrombocytosis). A positive sample had sequences with 99.8% homology for *Theileria ovis*. The Anaplasma-positive animals, showed mean values of: WBC (20.5±2.6 $x10^3$/µL; leukocytosis), Hct (26.2±1.8%), RBC (8.3±0.4 $x10^6$/µL; anemia) and PLT (722±150 $x10^3$/µL; thrombocytosis). In both infections, some animals also showed reactive lymphocytes and lymphocytic and erythrocyte inclusions in the blood smear. Regarding to fecal egg count, all animals showed a low grade of parasitism. Lastly, hyperechogenic (reactive) spleen and heterogeneous liver were visualized by ultrasound scan. All 16 animals were treated with three doses of oxytetracycline (20 mg/kg; IM) two days apart and clinical remission occurred. Nevertheless, a few animals demonstrated a long period of convalescence until productive rates returned.
Conclusions

it was described a clinical outbreak of hemoparasitosis caused by *Theileria ovis* and *Anaplasma* sp. in a meat sheep flock from southeastern Brazil with the first detection of *T. ovis* in Brazil. Veterinarian practitioners must be aware of these hemoparasitosis within the differential diagnosis list of diseases that affect sheep. This report also highlights the importance of further studies focusing on hemoparasites in small ruminants located at Brazil.

1773 - Growth performance and meat quality of lambs finished in confinement or on native grasslands with greater or lesser presence of legumes

Author: Luiza Rodegheri Jacondino , Cesar Poli, Raquel Raimondo, Jalise Tontini, James Muir

Objectives
Consumers are increasingly interested in getting quality meat from sustainable agricultural practices. In the southern region of Brazil, sheep meat production is characterized almost exclusively by production in pastoral systems, and the majority is present in the Pampa biome, one of the richest biomes in terms of biodiversity in the world, with 450 species of forage grasses and more than 150 species of legumes. This vegetation containing representatives of several botanical families produces a forage containing more secondary compounds, with emphasis on *Desmodium incanum*, a native legume with great amounts of condensed tannins and α-tocopherol (vitamin E), which have great potential for improving meat quality due to their antioxidant characteristics. This study investigated the effects of the finishing system and the presence of legumes in the diet on performance, carcass characteristics and meat quality.

Material and methods
Twenty-four castrated male Texel lambs with an average of 145 days of age and initial live weight of 28.36 ± 1.37 kg were randomly assigned into three treatments: (1) lambs finished in confinement (Confinement); (2) lambs finished in native grasslands with a low proportion of legumes (Less Legumes) and (3) lambs finished in native grasslands with a high proportion of legumes (More Legumes). After 82 days of exposure to dietary treatments, all animals were slaughtered with an average final live weight of 34.46 ± 1.33 kg and the *longissimus thoracis et lumborum* muscle was collected, vacuum packed, and stored at -30°C.

Results
Lambs finished on natural pastures had improved feed efficiency and meat with greater water retention capacity (WHC), higher α-tocopherol content and lower lipid oxidation after 9 days of storage ($P < 0.05$). Animals finished in native grasslands with a lower proportion of legumes (Less Legumes) had greater subcutaneous thickness fat (SFT) and meat with less cooking weight loss (CWL) and more attractive sensory characteristics (tenderness, juiciness, and flavor). Dietary treatments did not affect final pH, rib eye area, color descriptors, shear force, protein and lipid composition, γ-tocopherol deposition, and muscle cholesterol content ($P > 0.05$).

Conclusions
In conclusion, finishing lambs on natural pastures is an alternative to improve the quality and the oxidative stability of the meat, due to a significant increase in the deposition of muscle α-tocopherol and a decrease in the lipid oxidation after 9 days display. In addition to being an economic alternative, preserving and enhancing the sustainable exploitation of native pastures in the Brazilian Pampa, which is the second most devasted biome in the country. However, lambs grazing on native pastures with a higher proportion of legumes rich in condensed tannins did not promote an improvement in the nutritional quality of the meat.

1787 - Efficacy of Q Fever bacterin vaccines based on inactivated Phase I and Phase II Coxiella burnetii in sheep

Author: Tom McNeilly N, Sarah Williams-Macdonald, Mairi Mitchell, David Frew, David Ewing, Javier Palarea-Albaladejo, William Golde, David Longbottom , Alasdair Nisbet, Morag Livingstone, Clare Hamilton, Stephen Fitzgerald, Søren Buus, Emil Bach, Annemieke Dinkla, Hendrik-Jan Roest, Ad Koets

Objectives

Coxiella burnetii is an important zoonotic bacterial pathogen of global importance, causing the disease Q-fever in a wide range of animal hosts. In ruminant livestock, infections generally lack clinical signs prior to the onset of adverse reproductive outcomes, including birth of weak offspring, abortion, and infertility. Ruminants, and in particular sheep and goats, are the main reservoir of infection for humans, where symptoms of Q fever range from acute flu-like symptoms to persistent focalised infections. Vaccination is a key control measure to reduce both livestock and human infections. The only existing livestock vaccine Coxevac® (Ceva Santé animale, Libourne, France) is a killed bacterin vaccine based on the virulent phase I *C. burnetii* Nine-Mile strain. This has been licenced for use in goats and cattle since 2010, but until recently this vaccine was not licensed for use in sheep. The objectives of this study were firstly, to determine the efficacy of Coxevac® in sheep against *C. burnetii* challenge, and secondly, to determine whether an experimental bacterin vaccine based on an attenuated phase II *C. burnetii* strain, which would be safer to manufacture, was similarly effective.

Material and methods

Prior to mating, ewes (n=20 per group) were vaccinated subcutaneously with either Coxevac®, the phase II vaccine, or were unvaccinated. A subset of pregnant ewes (n=6) from each group was then challenged 151 days later (~100 days of gestation) with 10^6 infectious mouse doses of *C. burnetii*, Nine-Mile strain RSA493. Shedding of *C. burnetii* in faeces, milk and vaginal mucus was assessed during the periparturient period by quantitative PCR, and pregnancy outcomes recorded.

Results

Both vaccines provided protection against *C. burnetii* challenge as measured by significant reductions in bacterial shedding in faeces ($p = 0.030$), milk ($p < 0.001$), and vaginal mucus ($p = 0.002$), compared to unvaccinated controls. Furthermore, significant reductions in abnormal pregnancies (defined as abortions, or birth of still born or weak lambs) were observed in vaccinated groups compared to unvaccinated controls ($p = 0.008$).

Conclusions

This work highlights that the phase I vaccine Coxevac® can protect ewes against *C. burnetii* infection. This data has now been used to extend the license claim for Coxevac® to sheep in the UK and Europe. Furthermore, the phase II vaccine provided comparable levels of protection.

BUFFALO AND CAMELIDS

1178 - Improvement of fertility in Murrah buffalo (Bubalus bubalis) heifers in the non-breeding season in India

Author: Umesh Kumbhar, Tom Strydom

Objectives

The objective of the study was to evaluate the initiation and improvement of ovarian cyclicity and conception rate of Murrah buffalo heifers during the non-seasonal breeding period with hormonal intervention and hematological and mineral support. Murrah buffalo, a buffalo species from India is known for their seasonal breeding. Delayed puberty and sexual maturity in heifers are of the major reproductive problems observed in these animals.

Material and methods

The study was conducted during the non-breeding season (April & May 2018) in 16 apparent healthy, non-cyclic Murrah buffalo heifers in Mumbai, India. The animals were between 4 to 5 years old and weighing each approximately 350 kg. Prior to treatment, all the heifers were examined by rectal palpation three times a week to observe ovarian activity. No ovarian activity was observed in any of the animals.

Sixteen animals were selected for the study. Ten animals were allocated to Group I and 6 animals to Group 2 (control group). All the animals were maintained with the same management and feeding schedule. From Day 0 the animals from Group I were treated orally with a chelated mineral mixture at a dose rate of 70 g / animal / day for 21 days. The animals were also intramuscularly injected with a sodium salt of 4-dimethylamine, 2-methylphenyl-phosphonic acid 0.2 g (Tonophosphan®Vet, MSD Animal Health) at a dose rate of 10mg /kg body weight, on alternate days for 10 days. On Day 15 each animal was injected intramuscularly with buserelin acetate at a dose rate of 5ml per animal. No treatment was given to animals in the control group (Group 2).

All the animals were subjected to hematological and biochemical analysis. Ten milliliters (ml) blood was collected from each animal on Day 0 before any treatment was administered and again on Day 21 by venipuncture. One ml from each sample was transferred to a vial containing sodium citrate for hemo-analysis and the remaining blood was used for serum separation to analyze biochemical parameters. An automated hemo-analyzer was used for hematological analysis. Biochemical analysis included calcium, phosphorous and iron levels and were analyzed using standard diagnostic kits in a semi-automated biochemical analyzer.

Estrus signs in the treated animals were observed and estrus was confirmed by rectal palpation. Animals confirmed in estrus were artificially inseminated. Pregnancy of animals that were inseminated was confirmed by per rectal examination on day 60 post-insemination. Statistical analysis was carried out using the paired t-test as per Snedecor and Cochran (1994).

Results

Estrus was observed in all the animals of Group 1. Three animals showed estrus within 3 days and 7 animals within 6 days after treatment with Buserelin acetate in Group I, while only 1 animal in Group 2 showed estrus on Day 20 of the study.

The conception rate of the animals in Group 1 was 100%. Six animals conceived after the first insemination while 4 animals conceived after the second insemination during

the subsequent estrus. The one animal in Group 2 which was also inseminated when estrus was observed, was also confirmed to be pregnant.

Blood and biochemical parameters increased significantly in Group I post-treatment. The average hemoglobin from these animals increased from 9.48±0.74 g/dl to 11.88±0.90 g/dl (P< 0.05) between Day 0 and Day 21. The average serum iron concentrations from the animals increased from 99.60±2.57 µg/dl to 103.71±3.65 µg/dl (P< 0.01), the average serum calcium concentrations from 6.49±0.26 mg/dl to 9.59±0.35 mg/dl and average serum phosphorous concentrations from 3.61±0.20 mg/dl to 5.75±0.39mg/dl.

Changes in blood and biochemical parameters could be correlated with physiological observations in the animals. Mineral supplementations optimize the mineral profile in blood. For instance, an increase in hemoglobin and iron influences tissue oxygenation of the reproductive tract which could be responsible for follicular development. Buserelin acetate administration stimulates the hypophyseal pituitary axis and initiates the release of FSH leading to follicular development and ovulation. Ovarian development was well supported by an increase in serum calcium and phosphorous which both play a vital role in the conception rate of bovine species.

Conclusions

The therapeutic management protocol with chelated mineral supplementation, and treatment with sodium salt of 4-dimethylamine, 2-methyl phenyl-phosphonic acid 0.2 g and GnRH was found suitable for the induction of estrus and to enhance the conception rate in non-cyclic Murrah buffalo heifers during the non-breeding season in India. This study does not address the contribution of each treatment to the results and that this needs to be studied separately.

1209 - Surgical Management of Ileocecocolic Intussusceptions in Korean Native Calf

Author: namsoo kim , Dongbin Lee, Suyoung Heo, Hankyeong Lee

Objectives

Surgical Management of Ileocecocolic Intussusceptions
in Korean Native Calf

Material and methods

Signalment: A 2 month-old male Korean native calf was referred to the Jeonbuk Animal Medical Center, College of Veterinary medicine, Jeonbuk National University, because of prolonged diarrhea, depression and anorexia during 3weeks.

Results

Results: On the physical examination, abdominal distension presented in the right flank and percussion sound was audible. On the computed tomography (CT) and ultrasonography, round shaped lesion was observed in the area of large intestine. There were no reasonable findings on blood profiles. As the definitive diagnosis was not made, exploratory celiotomy performed to check the abdominal cavity. Between ventral midline and right flank was longitudinally incised because of easier approach and more reliable exploration. During surgery, there was found infarction lesion which looked like mass in the area of cecum and the small intestine was clear. The infracted lesion was revealed on intussuscepted intestines which included cecum, ileum and colon. The area of intussusceptions was excised and anastomosis was performed. In spite of intensive and supportive care, the patient died one day after surgical operation due to poor general condition.

Conclusions

Clinical Relevance: In clinic, the ileocecocolic intussusception is uncommon case compared within other intestinal intussusceptions. Most affected calves have a history of severe diarrhea and the prognosis is guarded result from the affected calves are usually in poor general condition. The definitive diagnosis generally knows from exploratory celiotomy. Because of that, early diagnosis is important for determining survival rate. If patients are diagnosed as soon as possible, good survival rate would be expected.

1223 - Study on pregnancy diagnosis in alpacas and llamas by analyzing estrone sulfate in fecal samples

Author: Thomas Wittek , Julia Baumeister, Elisabeth Müller, Anna Riepl

Objectives
Pregnancy diagnosis in South American camelids is usually done by transabdominal ultrasonography during early pregnancy. However, caused by the relatively high rate of embryonic mortality it is advisable to confirm if the animal is still pregnant during later pregnancy (e.g. 3rd to 5th month after mating). For this purpose, it would be ideal if the farmers could take a fecal sample and submit it to a laboratory for pregnancy check and parasitological examination at the same time.
The aims of this study were to measure the hormone estrone sulfate in fecal samples and to develop a non-invasive pregnancy detection method using the hormone estrone sulfate in feces for South American camelids. Estrone sulfate is synthetized by the placenta, and it has been shown that the serum concentration increases after placentation. Currently, estrone sulfate analysis for pregnancy diagnosis in South American camelids has been described using serum or urine, however urine and serum samples are more difficult to obtain for the farmers.
Material and methods
In this study, 86 mares were sampled over a period of one year, 81 were alpacas and 5 were lamas from different breeders in Germany and Austria. The mares were selected by the breeders without consideration of age, husbandry, nutritional status, or number of previous pregnancies. All mares were sampled on days 60, 90, 120 and 150 after mating, further 14 d before estimated parturition and 1 day after parturition. The fecal samples were analyzed in a professional laboratory (Laboklin) using a commercial Estrone-3-Sulfate (E1S) Competitive Elisa Kit (Thermofisher).
Results
Of the 86 mares, 83.72 % (72 mares) became pregnant and 16.28 % (14 mares) remained non-pregnant despite being mated. The concentrations of the estrone sulfate in feces increased in pregnant animals during pregnancy. However, the concentrations showed a wide variation between sampling days over the entire study period and between individual animals. The highest concentrations were reached 14 d before parturition. Additionally, large fluctuations in estrone sulfate concentration were also observed in the feces of on-pregnant mares. No significant differences of fecal estrone sulfate concentration were found between days 60 to 150 after mating between pregnant and non-pregnant animals.
Conclusions
The diagnostic value of estrone sulfate concentration to detect pregnancy was very low. It can be summarized that estrone sulfate analysis in feces is not a suitable method for pregnancy diagnosis in New World camelids between days 60 and 150 after mating.

1399 - EVALUATION BODY CONDITION SCORE OF SENIOR BUFFALOES

Author: Melina Yasuoka Marie

Objectives

The objective of monitoring buffalo body condition is to ensure the health, productivity, and well-being of the herd. This proactive approach allows for timely interventions, leading to improved reproductive performance, higher milk production, and overall economic efficiency in buffalo farming. Observing and understanding these changes in body condition is crucial for effective management of older animals.

Material and methods

The farm are located in the of Brotas city, São Paulo state, Brazil, had 1160 Murrah buffalos and were evaluated in March 2023. The body score assessment was identified with the animals brought from lots of farm paddocks, inspected one by one, contained 4-5 animals in the trunk hose/trough in single file, individually marked with a stick and paint, photographed and counted, and at the end of each batch, the animals returned to their paddock to start the next batch. The BCS methodology used was according to Alapati and collaborators (2010).

The visual assessment is observe the back and loin areas to assess the amount of fat cover. A well-conditioned buffalo should have a smooth, even covering of fat over these areas. The tailhead region look for fat deposits. In well-conditioned animals, the tailhead area should feel rounded and moderately padded.The on ribs run your hands along the sides of the buffalo's body, feeling the ribs. Ideally, you should be able to feel the ribs with slight pressure but not see them prominently. If the ribs are highly visible, the buffalo may be under-conditioned. Feel the pelvic bones. In a well-conditioned buffalo, the pelvic bones should not be overly prominent. Check the spinous processes along the back. They should not be sharp and easily visible in a properly conditioned animal. BCS is usually scored on a numerical scale, often ranging from 1 to 5 or 1 to 9, with higher numbers indicating better body condition (ALAPATI, et al.,2010).

Results

Assessing the body condition of buffaloes is an important aspect of their health and welfare evaluation Observe these changes in body condition is crucial for effective management of senior animals. Body condition scoring (BCS) is a common method used to assess the overall body fat and muscle condition of livestock, including buffaloes (De ROSA et al., 2005; MONTEIRO, et al.,2012).

In a total of 1160 buffaloes at Brotas farm, 115 animals were senile, 32 with a low body score condition (EC 1-2), and 83 in normal body score condition (EC 2.5-4). In the group below the score, there are senile cows that have given birth and are in prepartum. As the stage and animals age, it may be necessary to adapt feeding practices to meet their changing nutritional needs. This could involve providing more easily digestible feeds, adjusting the forage-to-concentrate ratio, or supplementing with specific nutrients.

Conclusions

A veterinarian can provide more specific guidance on body condition scoring and help tailor management practices to the unique needs of your buffalo herd. Observing and understanding these changes in body condition is crucial for effective management of older animals(WELFARE, 2009). Older animals are more prone to certain health

conditions, such as kidney disease, liver dysfunction, and metabolic disorders (TERRAMOCCIA, et al.,2005). These conditions can impact body condition and overall health. Regular monitoring and adjustments to feeding programs based on body condition can contribute to the overall welfare and productivity of the buffalo population.

1513 - Study of cell-mediated response during brucellosis and analysis of possible correlations with different Brucella tests in Mediterranean Buffalo (Bubalus bubalis)

Author: Anna Donniacuo Donniacuo, Esterina De Carlo, Giorgio Galiero, Lorena Schiavo, Maria Ottaiano, Roberta Brunetti, Piera Mazzone, Michele Napoletano, Vincenzo Bove, Roberta Vecchio, Alessandra Martucciello, Alfonso Gallo

Objectives

Brucellosis (BRC) is a zoonosis with a major economic and public health impact in many parts of the world, one of which is Southern Italy. Campania is the region with the highest concentration of buffalo farms, 1212 (source Italian National Livestock Database 2023). Typical Protected Designation of Origin mozzarella production accounts for about 20 % of the gross domestic product of the entire region with a value of 766 million euros in 2019.

In the buffalo species, Brucella infection is usually caused by *B. abortus* and less frequently by *B. melitensis*.

Up to now, diagnosis in ruminants is based on indirect serological tests according to the current EU Regulations 689/2020 and according to EURL and WOAH. The serological tests most frequently used are the Rose Bengal Test (RBT) and Complement Fixation test (CFT), tests that mainly detect anti-lipopolysaccharide smooth (S-LPS) antibodies. However, false positives may occur with both RBT and CFT due to cross-reactivity with other species of bacteria with S-LPS similar to those of Brucella spp.

The ELISA test can be used as serological test for brucellosis diagnosis.

Furthermore, also recognized among the official tests is the Brucellin skin test (BST)which is based on the cell-mediated (CM) response during brucellosis infection. The gamma-interferon test (**γ**-IFN test) is based on the same principle as the BST, but is developed in vitro.

Therefore, the objective of our study is to evaluate the use of different diagnostic tests for buffalo species.

Material and methods

In this preliminary study, for the evaluation of the iELISA test, serum samples from 80 animals were analyzed and divided into two groups: 32 animals from six BRC outbreak farms (PCR, RBT and CFT positive) and 48 samples collected from farms officially free of brucellosis. The iELISA test was carried out following the manufacturer's instructions [ID Screen ® Brucellosis Serum Indirect Multi-species, ID.vet, France].

For gamma interferon assay 170 samples from 15 BRC outbreak herds and 261 samples from 11 BRC-free farms were tested. Whole blood was stimulated with Brucellergene Ò OCB (100 U/well), Pokeweed Mitogen and PBS. Then followed the incubation for 16-24 hours at 37°C. IFN- **γ** levels were measured using a sandwich enzyme immunoassay (ELISA) following the manufacturer's instructions [Bovigam™, Life Technologies, Switzerland].

Results

The iELISA test confirmed the negative samples and the 32 positive reference samples.

The test also showed good repeatability both within and between runs: the average coefficient of variability (CV%) of intra-assay replicates was 7% with a 95% confidence

interval of 0.01-0.13 while the inter-assay CV% was 10% with a 95% confidence interval (0.03-0.17). An excellent level of agreement was observed (Cohen's K = 1).

All 261 buffalos from BRC-free herds were confirmed negative by RBT, CFT and **γ**-IFN test. Among 170 buffaloes from BRC-outbreak herds, 92.9% resulted RBT positive, while 83.5% resulted positive by CFT and 91.1% were positive to **γ**-IFN test. Out of these 155 **γ**-IFN test positive buffaloes, 146 were also positive to RBT and 135 were also positive to CFT, suggesting that **γ**-IFN test may have revealed an early stage of BRC infection in some of the animals, thus increasing the sensitivity of the diagnostic protocol.

Conclusions

The iELISA test, which provided the highest number of positive samples in the outbreak herd, could be considered as a useful test to accelerate the BRC eradication steps in problematic herds, although these preliminary results require further investigation of a greater number of samples. According to our data, the **γ**-IFN test could be used rather than the BST that is still a subjective and more time-spending test. It could be used in addition to the tests currently provided for BRC diagnosis, in particular to increase Sensitivity (use in parallel) and Specificity (confirmation test).

Implementation and validation of more available tests is a prerequisite for improving Brucellosis eradication programs, particularly for buffalo species, in which dedicated tests often do not exist.

1532 - Diagnosis of the current situation of buffalo breeding in the humid tropics of southeastern Mexico

Author: Raymundo Salvador Gudiño-Ecandón, Joaé Alfredo Villagómez Cortés, Jesús Hernández-Martínez

Objectives

Water buffalo production can have a positive impact on the economy of the diverse players in its production chain. In Mexico, buffalo farming offers trade opportunities at the national and international level, but the perceptions of different stakeholders are unknown. Therefore, the present study undertakes a diagnosis of buffalo situation in different production systems in the humid tropics of southeastern Mexico.

Keywords: Water buffalo, production chains, competivity, survey, stakeholders.

Material and methods

The field study was carried out from February to July 2023, collecting information in the states of Veracruz, Tabasco, Campeche, and Chiapas. Four surveys were applied to different stakeholders in the bovine production chain, namely: veterinarians, cattle farmers, buffalo producers and beef consumers. Snowball sampling was used to identify respondents. The results were analyzed by descriptive statistics.

Results

A total of 27 veterinarians were surveyed, 45% work with cattle, and only 50% have knowledge about buffaloes. The 83% considered that buffalo production in the southeast of Mexico can be adopted by ranchers, as it has advantages over cattle, such as its resistance to diseases and a better adaptation to tropical climates and terrain. Some 88% mentioned farmers´ lack of technical knowledge and the scarcity of resources as main challenges for buffalo breeding.

Twenty-six cattle farmers were surveyed to find out their level of knowledge and willingness to make a transition to raising buffalo on their farms. A 58% have low knowledge and 42% have medium knowledge about buffalo farming. The 90% have considered introducing water buffaloes on their farm, and of these, 38% cited lack of economic resources as the main reason for not starting this activity. The 91% are unaware of the comparative advantages of water buffaloes over cattle ranching and expressed their interest on learning the advantages of this activity.

Out of 18 buffalo ranchers surveyed, 43% have been engaged in the activity for less than 5 years and 57% for more than 5 years. They have their production in Veracruz (57%), Campeche (17%), Chiapas (16%), and Yucatán (10%). Buffalypso breed is the one with the greatest presence in Mexico. In the ranches of those surveyed, 42% had 5 to 49 buffaloes and 58% from 50 to more than 100. Beef production predominates (55%) over breeding stock (27%) and milk production (18%).

The 71% considered that buffalo farming is profitable, although it varies with operation size, production efficiency, feeding and management costs, products demand, and market prices. To boost growth and profitability of buffalo industry in Mexico, they considered it important to implement training and technical advice programs (57%), to secure financing (29%) and establish solid links among the different participants in the buffalo production chain (14%). Most surveyed consumers eat beef at least once a week, and a smaller percentage consume it rarely.

The 27% claimed to have eaten buffalo meat; a 47% know buffalo meat but have not tried it; 27% do not know it. Some 67% would try buffalo meat and 13% would be willing to pay a higher price if it is shown that it has more nutritional benefits than beef. Cheese and yogurt are the most consumed buffalo´s dairy products.

Conclusions

Buffalo production can be feasible for ranchers with land not suitable for bovine cattle, also for those with large tracts of land, and those who have experience managing cattle. The adaptability of buffaloes to tropical climate and its presence in places with favorable ecosystems offer favorable perspectives to take advantage of buffaloes. The main problem that was identified was the lack of consumer knowledge about the nutritional benefits of products derived from buffaloes; However, all stakeholders recognize that this livestock farming is expanding, and they consider it valuable for environmental conservation. Consumers are willing to explore healthier diet alternatives and taste buffalo products.

1581 - The feasibility of creating a buffalo system-product committee in Mexico

Author: Raymundo Salvador Gudiño Escandón, Jesús Hernández-Martínez, José Alfredo Villagómez-Cortés

Objectives
A system-product is a mechanism for planning, communication and permanent consultation between the economic actors participating in production chains. The system-product committees promote the positive development of agriculture and livestock industries. Currently, there is no system-product committee for the breeding and production of buffaloes in Mexico. Therefore, the objective of this study was to analyze the perspectives for the creation of a buffalo system-product committee in Mexico.
Material and methods
The research was documentary in nature and focused on the search for information on the legislation that regulates the creation and operation of system-product committees in Mexico. Then, information was sought about the development and current situation of buffalo breeding in Mexico, and the organization for the breeding and promotion of the species. Together, these perspectives allowed to gain a comprehensive overview of the feasibility and viability of establishing a system-product committee for the breeding and production of buffaloes in Mexico.
Results
A production chains (or system-product as is called in Mexico) is a set of elements and concurrent agents of the productive processes for agricultural products, including technical equipment supply, productive inputs, financial resources, as well as collection, transformation, distribution, and marketing activities. In Mexico, the Sustainable Rural Development Law states that the central objective for creating system-product committees is to plan and organize production, promote the improvement of production, productivity and profitability. For each basic or strategic product, a single national product-system committee will be established and granted autonomy to self-define and limit itself; nevertheless, a number of regional system-product committees might be created as required. Nowadays, there are 33 national product-system committees, and 242 state product-system committees in operation. The system-product committees are the organizational entities responsible for the design, the implementation, the evaluation and follow-up of actions reinforcing all and every one of the system's links. In the long term, it is expected that all economic agents participating in the production chain obtain positive margins.

Among the functions performed by a system-product committee are: promoting the adoption of sustainable practices; to establish quality and safety standards for production and marketing products; to promote innovation, research, training and educational programs on topics relevant to the productive sector; to establish communication and collaboration mechanisms between the different actors and stakeholders in the productive sector; and to represent the interests of the productive sector before the authorities and other relevant actors at the national and international level.

Water buffalos were first introduced to Mexico in the 1970s. The buffalo population is estimated at about 50,000 head. In 2006, the Mexican Association of Buffalo Breeders (AMEXBU) was established, which is recognized by world organizations such as the International Buffalo Federation (IBF). Amexbu can be the spearhead to establish the national committee, since it brings together breeders and they can extend an invitation to incorporate other agents involved directly or indirectly in buffalo production. Since the buffalo system product committee must be a space for coordination and collaboration between the different actors in the sector, including producers, processors, marketers and consumers, its creation would facilitate the exchange of information, the design of joint strategies, the resolution of common problems, the improvement in products quality and efficiency in production processes.

Conclusions

The creation of a buffalo product committee-system is feasible because Mexico has the necessary socioeconomic and political conditions. Establishing a product system committee for buffalo would be a strategic measure that would harness the potential of this species, strengthen the sector, improve product quality, and could increase profitability for producers.

Keywords: Water buffalo, farmers' organization, production chains, competitiveness.

1674 - Development of novel herbal formulation from Amazonian plants for wound healing in Buffaloes

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Objectives

We evaluate the effectiveness of herbal formulations based on Murumuru (*Astrocaryaum murumuru*) butter, either alone or in combination with ethanolic extract of jucá (*Libidibia ferrea*), copaíba oil (*Copaifera reticulata*) and andiroba oil (*Carapa guianensis*) for wound healing in buffaloes.

Material and methods

Six healthy Murrah buffaloes were used, in which six excisional dermal lesions were induced. The wounds were superficial and made with a 10 mm punch after local anesthesia, three on each side of the animal, 20 cm below the spine, and 25 cm apart from each other. A total of 18 wounds were used to evaluate wound healing, distributed homogeneously among treatments and animals to limit the effect of individual variations on healing results. Twenty-four hours after the induction of dermal lesions, each wound was treated daily and exclusively according to the following groups:

Control: commercial ointment Alantol® (allantoin 3.0 g, zinc oxide 3.0 g, and excipients q.s.p 100 g).

Murumuru butter: A crystalline liquid system, as detailed in patent application BR 1020150308884, comprising 40% Murumuru (*Astrocaryum murumuru*) butter, 40% Surfactant, 5% Propylene glycol, and water.

Herbal mixed compound (HMC): Utilizing the Murumuru butter base, this formulation incorporated 2.5% ethanolic extract of Jucá, 2.5% Copaíba oil, and 2.5% Andiroba oil.

Each wound received 0.5 ml of the assigned formulation daily for a 21-day duration. Wounds were evaluated on days 1, 3, 7, 14, and 21 post-induction, employing caliper measurements and digital photographic documentation. The wound diameter was obtained by measuring the biggest distance between the edges of the wounds and the wound retraction was calculated according to the formula: RI = 100 × (W0 - WI) / W0, where: IC: retraction index (%), W0: area initial wound area (D0), WI: final area (D21). To evaluate buffalo health, blood was sampled for hematological analysis to check RBC (red blood cell count, HGB: hemoglobin, HCT: hematocrit; WBC: White blood cell count). The skin temperature of each lesion was measured daily using an infrared thermometer pointed to the center of each wound. For statistical analysis, a general linear model was used for each of the response variables: wound diameter, wound retraction, and skin temperature, considering the Animal as a covariable and the fixed factors Day and Treatment and their interaction, followed by means comparison by Fisher's Least Significant Difference (LSD) method.

Results

Hematological analyses carried out throughout the study indicated that the animals remained healthy with values within the limits for the species: RBC 6.7 to 11.8 x10^6 /µl; Hemoglobin: 10.7 to 13.5 g/dL; HCT: 35 to 45%; WBC 5.4 to 12.4 x 10^3 /µl. Regarding temperature, a difference ($p<0.05$) was observed with a higher overall skin temperature

in the Murumuru butter (36.7ºC) and HMC (36.5ºC) groups compared to the control group (36.3ºC). At D21 the control group had a mean of 0.61 ± 0.93 cm of wound diameter, which was higher (P<0.001) than the Murumuru butter and HMC groups that showed values near zero. A similar pattern was observed for the retraction index, with HMC group heaving a healing rate of 100%, indicating complete efficacy, being similar to the Murumuru group with retraction of 99.9% ± 0.02, both higher (p<0.001) than control group, which, despite using a commercial formulation with high added value, had a retraction of 93.9% ± 9.25.

Conclusions

Based on wound diameter and retraction rate, herbal formulations derived from Amazonian plants showcased superior wound healing attributes, outperforming the commercial allantoin-based product. In this study, the addition of Jucá, Copaíba, and Andiroba to the Murumuru butter did not promote additional wound healing benefits, possibly due to the limited wound size and the high speed of dermal healing in buffaloes. A more challenging clinical study is required to better evaluate the possible benefits of the herbal mixed compound formulation in this species.

PUBLIC HEALTH, FOOD QUALITY AND TRACEABILITY

1133 - Assessment of the in vitro sensitivity of enterobacteria collected from fecal samples of young calves treated or not with paromomycin

Author: Yves Millemann, Pedro Rodriguez Fernandez, Clara Bourel-Conroy, Geert Vertenten, Romane Dubost, Siddartha Torres

Objectives

Cryptosporidiosis, caused by *Cryptosporidium parvum*, is a severe disease of young calves resulting in diarrhoea, weight loss, and occasionally mortality. In France, halofuginone and paromomycin are used orally to control the disease. Halofuginone belongs to the quinazolinone derivatives group and is an antiprotozoal agent specifically designed for the prevention and treatment of diarrhoea associated with cryptosporidiosis. On the other hand, paromomycin is an aminoglycoside antibiotic used only to treat (and not prevent) diarrhoea. Oral administration of paromomycin to calves may result in the selection of resistance to aminoglycosides and other antimicrobial agents in bacteria present in the gastrointestinal tract of exposed animals. The objective of this study was to compare the *in-vitro* sensitivity to antimicrobials of bacteria isolated from the faeces of young calves exposed or not exposed to paromomycin, as well as calves treated or not treated with paromomycin.

Material and methods

Ten farms, representative of the French beef cattle production, were included in the study. In five farms, young calves were systematically exposed to paromomycin for the treatment of diarrhoea (PR farms) whereas other five farms did not use paromomycin (or any other aminoglycosides) for the treatment of diarrhoea (non-PR farms).

In PR farms, the faeces of 5 calves treated with paromomycin for diarrhoea during the previous week were sampled for on Day 0 (D0) and then 2 months later (D60). From the same five farms, faeces from 5 calves not treated with paromomycin were also collected on D0 and D60. In the five Not-PR farms, the faeces of 5 calves of approximately the same age, were also collected with the same sampling schedule.

Escherichia coli and *Enterococcus* spp. isolates were obtained from the faecal samples and subjected to antibiograms for 15 antimicrobials and Etest® (bioMérieux, France) values for paromomycin.

Results

In total, 25 paromomycin-treated and 24 paromomycin-not-treated calves from PR farms, and 25 calves from non-PR farms were sampled. Antibiograms and Etest® values for paromomycin of a total of 1,435 *Escherichia coli* and 184 *Enterococcus* spp. isolates were analysed.

At D0, the proportion of the highest paromomycin Etest® value category (>1024 mg/L) increased with the paromomycin exposure, from 0.1% in calves of non-PR farms, 10.2% in not-treated calves in PR farms and 20.1% in calves that were treated with paromomycin in PR farms. This proportion drastically decreased at D60, to 0%, 1.8% and 0.6% respectively.

In *Enterococcus* spp. isolates, the proportion of the highest paromomycin Etest® value category (>1024 mg/L) at D0 also increased with paromomycin exposure, from 8.9% in

calves of non-PR farms, to 22.8% in untreated calves of PR farms and 48.5% in treated calves from PR farms. This proportion decreased at D60 except in the not-treated calves from PR farms.

Regarding *E. coli* isolates collected at D0, paromomycin treated calves had the highest proportion of resistance for most antibiotics (neomycin, gentamycin, all β-lactams, oxalinic acid, flumequine and tetracycline) except for florfenicol, marbofloxacin and enrofloxacin, when compared to the isolates from not treated calves from PR farms. The most important resistance was observed in calves treated with paromomycin, with at least 50% resistant isolates: neomycin (74%), amoxicillin (87%), trimethoprim-sulfamethoxazole (54%) and tetracycline (67%). At D0 and D60, the proportion of resistance was very low or negligible in calves from non-PR farms in comparison to calves from PR farms.

However, *Enterococcus* isolates collected at D0 from treated and not-treated calves from PR farms presented a very high resistance proportion for almost all antibiotics tested: > 60% for kanamycin, streptomycin, cephalexin, oxacillin-Streptococcus, lincomycin, tylosin, erythromycin and tetracycline. Isolates of non-PR farms presented the lowest resistance proportion compared to isolates of the PR farms. At D60, antimicrobial resistance was still high in PR farms.

Conclusions

In this study, the systematic use of paromomycin for the treatment of diarrhoea of young calves resulted in a higher proportion of *in-vitro* resistance of enterobacteria to aminoglycosides and other antibiotics and it may lead to an increased risk of antimicrobial resistance of bacteria present in the gastrointestinal tract of young calves.

1355 - The presence of bovine tuberculosis in certified regions of the north and northeast of Rio Grande do Sul underscoring the sanitary and economic impact in a major dairy basin in Brazil

Author: Renan Lazzaretti, Janine Andreia Surkamp, Rafael Backes, Alan Issa Rahman, Bianca Ribeiro de Souza

Objectives

Bovine tuberculosis is an infectious disease, caused by *Mycobacterium bovis*, which mainly affects cattle, but can affect other animal species, besides being a zoonosis. In cattle, tuberculosis causes damage to various organs, causing weight loss, affecting the respiratory system and especially the immune system, harming animal production and consequently the economy. Due to these and other problems, bovine tuberculosis is considered a major public health problem in countries such as Brazil, where the concern occurs mainly in regions such as the north and northeast of Rio Grande do Sul, places with a large concentration of dairy production, activity that generates direct daily contact between cattle and humans. Therefore, the control and prevention of this disease becomes fundamental in this region, where our team works isolating outbreaks and certifying properties as tuberculosis-free, through specific tests, by controlling the movement of cattle and the adoption of basic biosecurity practices that promote a pact focused on one health, encompassing healthy cattle and safety for both rural producers and the society that consumes milk from this region. The objective of the present work was to determine the prevalence of bovine tuberculosis in the period from January to December 2022 in herds tested in the north and northeast region of Rio Grande do Sul, where works the veterinary team of the agricultural cooperative responsible for a program of control and eradication of this disease that is so important for the economy and public health of the region.

Material and methods

Thus, data from tuberculosis tests carried out in 20 municipalities were used, where 5 animals presented positive results to the tuberculin skin test. The evaluation method used in the herds was the Comparative Cervical Test, which is considered a confirmatory method for bovine tuberculosis. This test is carried out in the region of the spine of the scapula, starting with knitting two points in the area, and continuing with measuring the skin fold in each area using a cutimeter. After such procedures, the Purified Protein Derivative (PPD) is inoculated intradermally, 0.1ml of derivate tuberculin from *Mycobacterium avium* (cranial to the spine of the scapula) and 0.1ml of derivate tuberculin from *Mycobacterium bovis* (caudal to the spine of the scapula) with a distance of 15 to 20 cm between them, with the reading and interpretation of results carried out 72 ± 6 hours after inoculation of the PPDs.

Results

After the test and the definitive result was obtained, the animals diagnosed positive for bovine tuberculosis were marked with the letter P (positive) with a burning iron on the right side of the face, and sent to slaughter. The information obtained about the animal population reached and the number of positive animals was obtained from the database of the cooperative in question, and the results were displayed in a spreadsheet using the PivotTable tool in the Microsoft Office Excel 2017 software. In this context, it was observed that of the 7631 animals tested, only 5 animals were diagnosed positive for

tuberculosis, that is, a prevalence of 0.065%, a significantly low value when compared to other regions of the country. On the other hand, approximately 80% of the properties where the tests were carried out already had previous certification tests and were considered free of tuberculosis until now, which alerts us to the circulation of the disease, as positive cases were found in the region, and ratifies the difficulty of eradicating this disease.

Conclusions

Because tuberculosis presents great economic losses, since animals tested positive are slaughtered and the rest of the herd that has been in contact with these animals stays at imminent risk of acquiring the disease, and it is also considered a public health, due to its zoonotic nature. That way, it is extremely important to continue with this works of quantification and monitoring cattle herds and raise awareness of the economic and health serious problems attached to this disease and its importance in prevention among cattle producers.

1574 - Somatic cell concentration in milk processed by traditional rural cheese agroindustries from Aculco, State of Mexico

Author: Randy Alexis Jiménez Jiménez, Mauricio Miguel-Estrada, María Camila Rendón-Rendón, Luis Manuel Chávez-Pérez, Valentín Efrén Espinosa-Ortiz

Objectives
Milk is a strategic food for human nutrition and the economy of the cheese industry in Mexico. It is important to evaluate hygiene indicators of milk intended for cheese production because these can influence the production, performance and flavor of the cheese, in addition to providing information on the production process and management of the milk. The pourpose of the study was to determine the hygienic quality of milk processed in cheese agroindustries by counting somatic cells in order to identify compliance with the standards established by the Mexican standard NMX-F-COFOCALEC-2012.
Material and methods
During the fall season, October to December 2022, 51 samples of raw milk received in three rural agroindustries producing traditional cheeses in the town of Las Lajas, Aculco, State of Mexico were studied. Somatic cell counting was performed using the De Laval Cell Counter equipment.
Results
It was found that the milk processed by the agroindustries studied presented an average somatic cell concentration of 534,471 ± 518,400 cs/ml, which placed the milk in class 3 according to the Mexican standard NMX-F-700-COFACALEC- 2012. 49.02% of the processed milk was found in class 1 (<400,000 cs/ml), 3.92% in class 2 (401,000 - 500,000 cs/ml), 21.57% in class 3 (501,000 - 749,000 cs/ml), 9.80% in class 4 (750,000 - 1,000,000 cs/ml) and 15.68% outside the limits allowed by the standard (>1,000,000 cs/ml).
Conclusions
The milk received in the agroindustries of the town of Las Lajas, on average, met the hygienic standards allowed by Mexican regulations; however, there were milk producers who have a somatic cell count in the milk that exceeded the permitted, indicating that it is not suitable for consumption; this could be detrimental to various actors in the production and economy of the local cheese agri-food chain, making it important to improve hygiene in milking, monitor and attend to cases of subclinical mastitis.
The study was funded by PRONAII-CONAHCYT 321289: “Desarrollo de estrategias participativas para el fortalecimiento de redes de producción y consumo de productos lácteos tradicionales orientadas a la soberanía alimentaria de territorios del centro-occidente de México.”

Animal behavior and welfare

1012 - Novel wound management strategies for dehorning wounds in cattle

Author: Samantha Rudd .

Objectives

Dehorning is a routine animal husbandry procedure performed to remove horns of cattle for reduced risk of injury to handlers, other animals, and the animal itself. The removal of horns reduces carcass bruising, estimated to cost the Australian cattle industry millions of dollars annually. Amputation dehorning induces a significant amount of pain evident through behavioural and physiological abnormalities, as well as haemorrhage, infection, and myiasis, leading to morbidity and mortality (Mainau, Temple & Manteca, 2012). Calf mortality directly resulting from dehorning has not been quantified, however, a mortality rate of 2.1% from branding to weaning was observed in calves that had been dehorned (Bunter *et al*, 2013). Post-operative haemorrhage and infection have been suggested as the main factors causing mortality in dehorned calves (Fordyce, McMillan & McGrath, 2018). Treatments available for dehorning include local anaesthetics (LAs) and non-steroidal anti-inflammatory drugs (NSAIDs) which address short-term pain but are limited in their ability to address longer-term pain and other post-operative complications. Additionally, these products provide no aid or protection to the wound, leaving them vulnerable to haemorrhage and infection.

The application of a dressing to dehorning wounds have been found to reduce haemorrhage and acute infection (Fordyce, McMillan & McGrath, 2018). This project aims to evaluate the application, adherence, and efficacy of two novel wound treatments, a polycaprolactone (PCL) patch and a hydrogel dressing, and an existing haemostatic wound treatment Vetigel® (Cresilon Inc).

Material and methods

Nine weaned horned *Bos taurus* calves aged approximately 6 months were used in this pilot study, allocated to one of three treatment groups undergoing amputation dehorning, blocked by horn diameter: PCL patch (PCL), hydrogel dressing (HG), or Vetigel (VG). Calves were loaded into the crush and administered local anaesthetic lignocaine (Illium Lignocaine 20; 6ml per horn) to establish a cornual nerve and ring block to each horn and a subcutaneous injection of meloxicam (Metacam® Boehringer Ingelheim) 20 minutes prior to dehorning. At this time, calves were allocated a number, painted on both flanks using tail-paint (Leader Products Pty Ltd, Craigieburn, VIC), and horn diameter was measured at the junction of the skin and horn at the widest part, to allow treatment groups to be allocated. Calves were re-loaded into the crush after 20 minutes and dehorned using medium scoop amputation dehorners. Wound diameter was measured immediately post-excision using digital callipers and a digital image was taken on the wound. At this time, the presence of arterial spurting and the presence of an open sinus were recorded (0=no, 1=yes). The allocated treatment was then applied, being timed to determine ease of application. The PCL patch was applied by applying light pressure on the patch over the wound for 15 seconds to allow blood to soak through to aid adherence. The hydrogel patch was applied to the wound with light

pressure applied for 15 seconds to ensure adherence. The Vetigel treatment was applied by firstly lightly dabbing the wound with sterile gauze to remove excess blood. The VG treatment was then administered via syringe at a perpendicular angle to the skin into the wound cavity. A wet sterile gauze was then used to apply light pressure to the treatment to aid adherence. Calves were then released from the crush, and adherence of treatments upon release were recorded as 0 or 1, and degree of haemorrhage was measured as 1-3, depending on the proportion of the jaw from poll to mandible covered in blood (1= cranial third, 2 middle third, 3 distal third). Adherence of treatments and degree of haemorrhage were measured as the animal left the crush, then at 6-hours post-dehorning, via visual observation in pens.

Results

Results are not yet available as this project will take place in the weeks following abstract submission. Results will be provided ASAP upon completion of the project.

Conclusions

The outcomes of this study will inform the future direction of the greater project, identifying potential modifications or issues with the novel and existing products.

1027 - Plasma concentrations of meloxicam in dairy calves fed medicated pellets

Author: Sabrina Lomax, Chloe Wilson, Dominique Van der Saag, Peter White

Objectives

Meloxicam is a commonly administered NSAID that has proven efficacious in reducing pain related behaviours and the physiological responses associated with dehorning. The duration of action of a single oral administration of meloxicam is an average of 27 h, however, pain associated with surgical procedures often persists beyond the duration of action of a single dose. Administering multiple doses of a NSAID is impractical for most commercial farms, particularly larger extensive operations. Repeated treatment requires additional labour and handling of animals, which can cause stress and damage to wounds, impeding the healing process, and adding to operation costs. The use of medicated pellets (feed concentrate) provides the option of drug delivery prior to a surgical procedure, improving pain mitigation through prevention of the inflammatory cascade. Quantifying the plasma concentrations of a medicated feed, will enable pre-emptive analgesia to be administered to align with concentrations known to provide analgesia, thus providing therapeutic relief prior to tissue injury.

The aim of this study was to evaluate whether a MMP ration fed to calves would provide a method for delivering sustained analgesia over time. This was achieved through quantifying the plasma concentrations of meloxicam in calves fed a meloxicam medicated pellet (MMP) ration for 7 days.

Material and methods

Twelve Holstein Friesian calves (avg 85 kg ± 18.7 kg SD) were offered a MMP feed ration as a group, with meloxicam administered at an approximate dose rate of 1mg/kg of bodyweight per day. One kg per calf of 22% medicated pellets were fed to calves in two feed troughs each morning between 8-9am for 7 days. On days 8 and 9, calves were fed 1 kg per calf of non-medicated 22% calf grower pellets in order to capture the meloxicam elimination phase.

Blood samples were collected by jugular venepuncture prior to the first treatment and then daily for 9 days. Plasma meloxicam concentrations were determined by HPLC analysis with UV detection.

The concentration of meloxicam in the pellets was tested on six occasions. The mean +/- SD of meloxicam concentration per gram of pellet was 0.043 mg/g pellet +/- 0.052 mg/pellet.

Results

Plasma concentrations of meloxicam in MMP treated calves reached a maximum of 3.81 ug/mL (avg 2.53 ug/mL ± 1.05 ug/mL SD) within 24 h and increased daily, reaching a maximum concentration of 6.95 ug/mL (avg 4.07 ug/mL ± 2.17 ug/mL SD) on Day 6. By day 9 (following elimination) the average plasma concentration was 0.68 ug/mL.

These plasma concentrations are comparable with those in studies where calves were adminstered meloxicam orally and intravenously.

Conclusions

The findings of this study indicate the potential to extend duration of action of meloxicam through feeding MMP to calves. However additional research is required to determine clinical efficacy. Plasma concentrations demonstrate that MMP provided consistently high circulating concentration of meloxicam to calves across 1 week, as compared to a single dose of injectable meloxicam. This method of administration, feeding meloxicam in pellet form, has the potential to minimise the associated stress, labour and handling of conventional administrations, proving to be a practical option for longer duration pain mitigation. Further investigation has been conducted to determine the efficacy of the pellets to deliver sustained therapeutic relief following invasive husbandry procedures.

1087 - Characteristics of the ideal treatment, and thoughts on pain relief, for clinical mastitis in dairy cows

Author: Greg CHAMBERS, Matthew WELLS

Objectives
Efforts to optimise clinical mastitis treatment protocols may focus on efficacy, animal welfare, productivity, food safety, and antimicrobial stewardship, which require behaviour change. Understanding the thoughts, beliefs, and attitudes of producers and themselves is expected to help animal health advisors engage and communicate more effectively with their producers and improve how they can work together to optimise clinical mastitis treatment. The objectives of this study were to firstly determine why New Zealand producers and veterinarians make the choices they do for selecting or recommending clinical mastitis treatments and, secondly, ascertain their views on providing pain relief for cows with clinical mastitis.
Material and methods
In December 2022, six workshops in three regions of New Zealand were conducted, with one producer and one veterinarian workshop conducted in each region. Researchers recruited producers and veterinarians from one veterinary clinic in each region (Waikato, Bay of Plenty, and Canterbury). The clinics did not randomly select Veterinarians who provided services to dairy producers, and dairy producers who were customers of the same clinics were randomly selected from each clinic's database. The workshops were approximately two hours long and asked participants to firstly, identify and explain the characteristics of the ideal treatment for clinical mastitis and secondly, share their thoughts about pain management for clinical mastitis cases. The collection of ideas developed into themes through facilitative questioning and ranked in importance by voting. Researchers collected written, audio, and photographic data and subjected them to content analysis, a qualitative approach to exploring the thoughts participants had. Researchers also assigned characteristics of the ideal clinical mastitis treatment a score of 10/rank, and the sum of the scores was added.
Results
Fifty-one people (27 producers and 24 veterinarians, including one technician) attended the six workshops. Producers and veterinarians identified ten unique characteristics of the ideal clinical mastitis treatment. "Effectiveness" had the highest score overall and covered factors like animal welfare, productivity, reduced risk of compliance errors and audit problems, and reduced selection pressure for antimicrobial resistance (AMR). "Short milk withdrawal period" was the second highest scoring characteristic and was the most prevalent characteristic with producers, mainly to manage the risks of non-compliance and residues. At the same time, "effectiveness" was the most popular amongst veterinarians. "Single dose" and "user friendly" were the third and fourth highest-scoring characteristics, respectively. Compliance was a very familiar theme throughout all workshops; both vets and producers showed the popularity of a user-friendly product. Both groups believed that anti-inflammatories improve treatment efficiency. Veterinarians emphasised how much work they did to grow pain relief uptake and noted some barriers (gender, age, and communication). Producers indicated that adding pain relief adds complexity, and the combination with the antibiotic was ideal.
Conclusions

Our workshops with New Zealand producers and veterinarians indicated that the ideal clinical mastitis treatment combines high efficacy short milk withholding time, is ideally a single dose product, and is user-friendly. For the cows' productivity, the perception of pain relief is to be entirely beneficial. Pain relief/anti-inflammatories should be in combination with the mastitis treatment.

1101 - Effect of Bovine Appeasing Substance on parameters productive, immunological and cortisol plasma levels of multiparous Holstein cows in the transition period

Author: Maria Carolina de Araújo Narval, Milene Lopes dos Santos, Leonardo Marins, Uriel Secco Londero, Antônio Amaral Barbosa, Nathaly Ana Carpinelli, Osvaldo Sousa, Francisco Augusto Burkert Del Pino, Rodrigo de Almeida, Viviane Rohrig Rabassa, Marcio Nunes Corrêa

Objectives
The aim of this study was to determine the influence exerted by the Bovine Appeasing Substance (BAS) on milk yield, food intake, parameters immunological and cortisol plasma levels in multiparous Holstein cows in the transition period.
Material and methods
This study included 24 multiparous Holstein cows (n=24), who underwent monitoring from day 28 pre-partum to day 21 post-partum. Adopting the completely randomized design, the animals were distributed into two groups: control (n=12) and BAS (Secure Cattle®, Nutricorp, Araras, SP, Brazil; n=12). First, each animal was given 5 mL of the product via topical administration into the nape on days 28 and 14 pre-partum and then on the day of the calving. In the case of the control animals there was no the application of the placebo. The food intake was assessed every day using smart feeders. The milk yield was determined electronically. The blood samples were drawn on days 28 and 14 pre-partum, the day of the calving, and also on days 7, 14, and 21 post-partum. These samples were analyzed for myeloperoxidase (MPO), substance P, paraoxonase 1 (PON-1) and cortisol.
Results
The statistical analyses were all done with the JMP Pro 14 software, the accepted level of significance was $P \leq 0.05$. It was evident that the cows that were given the BAS showed higher milk yield, revealing an increase of 2.92±0.53 kg/day in comparison with the control group (29.04±0.53 *vs* 26.12±0.53; $P<0.05$). The Dry Matter Intake (DMI) during the pre- and post-partum periods was greater for the control group. The data recorded was 14.31±0.40 kg/day for control group versus 10.34±0.34 kg/day for the animals belonging to the BAS group ($P<0.05$) during the pre-partum period and 16.24±0.44 kg/day for control group, while the BAS group registered 12.91±0.41 kg/day ($P<0.05$) during the post-partum period. In terms of the biochemical analyses, the BAS group have lower levels of post-partum plasma cortisol (15.70±1.07 control and 11.58±0.92 BAS; $P<0.05$), although the other markers remained unaffected.
Conclusions
Multiparous Holstein cows during the transition phase and subjected to the BAS treatment show decreased cortisol plasma levels in pospartum, higher milk yield and reduced DMI.

1207 - Concordance levels of routine and confirmatory bovine brucelosis tests in São Paulo State between 2020 and 2022

Author: Rodrigo de Souza Ferreira, Klaus Saldanha Hellwig, Eduardo Lipparelli Fernandez, Flávia Vasques, Beatriz Bassora Paim, Hugo Leonardo Riani Costa, Rodrigo de Lemos Marini, Affonso dos Santos Marcos, Izabelle Mariane Cordeiro, Ricardo Spacagna Jordão, Ana Paula Cunha Belchior

Objectives

Brucellosis is a transmissible infeccious disease that affects bovines, other animal species and humans, wich makes it a relevant zoonosis in public health. In bovines and bubalines the disease is caused by *Brucella abortus*. Bacteria of the genus *Brucella* are a facultative intracellular parasite, non-motile gram-negative coccobacillus. The main clinical signs in animal hosts are joint inflammation (arthritis), testicle inflammation (orchitis), recurrent abortion, retained placenta and vaginal discharge. The disease control methods in bovine and bubaline herds are based on mandatory vaccination of all female calves between 3 and 8 months of age, in addition to mandatory diagnostic test performed on animals before transport to any animal gathering events, on animals designated to reproduction and also on milk and milk derivated processing stablishments milk suppliers. This study aims to verify the concordance level between the routine and confirmatory brucellosis tests.

Material and methods

The routine tests are perfomed by veterinary doctors licensed by Ministério da Agricultura e Pecuária (MAPA) and in event of a positive result the veterinarian can submit the same serum sample to undergo a confirmatory test performed by any laboratory certified by MAPA. It is mandatory for licensed veterinarians to report all tests results in Sistema de Gestão de Defesa Animal e Vegetal (GEDAVE), an online system administered by Coordenadoria de Defesa Agropecuária. All the data used for this analysis was extrated from GEDAVE.

Results

In 2020, 138 bovines with positive results in Rose Bengal test (routine test) had their positive serum submited to the 2-mercaptoethanol (confirmatory test), with 100 positive results and 38 negative, thus a 72,46% concordance value (number of animals with positive results in both routine and confirmatory brucellosis tests). In 2021, 74 bovines with positive results on routine test were submitted to the confirmatory test, resulting in 26 positive and 48 negative results in 2-mercaptoethanol, a concordance value of 35,14%. In 2022, 84 bovines with positive results in Rose Bengal test were also submitted to confirmatory test, wich resulted in 55 confirmed positive and 29 negatives, a 65,48% concordance value.

Conclusions

The average concordance value for the three years period was 61,15%, which infers the importance of the confirmatory test to prevent the elimination of false positives on routine bovine brucelosis tests.

1208 - Concordance levels of routine and confirmatory bovine tuberculosis tests in São Paulo State between 2020 and 2022

Author: Rodrigo de Souza Ferreira, Klaus Saldanha Hellwig, Flávia Vasques, Ricardo Spacagna Jordão, Ana Paula Cunha Belchior, Affonso dos Santos Marcos, Eduardo Lipparelli Fernandez, Rodrigo de Lemos Marini, Beatriz Bassora Paim, Hugo Leonardo Riani Costa, Izabelle Mariane Cordeiro

Objectives

Tuberculosis is slow progressing, transmissible, infeccious disease that affects bovines, other animal species and humans, wich makes it a relevant zoonosis in public health. In bovines and bubalines the disease is caused by *Mycobacterium bovis*. Lesions due to the disease can be found in lungs, liver, spleen, udder and carcass. The main clinical signs in animal hosts are weight loss and cough, although it can be unnoticed by breeders. The disease control methods in bovine and bubaline herds are mandatory diagnostic test before new acquisitions to the herd, performed on animals before transport to any animal gathering events, on animals designated to reproduction and also on milk and milk derivated processing stablishments milk suppliers, following by destruction of positive animals. This study aims to verify the concordance level between the routine and confirmatory tuberculosis tests.

Material and methods

The routine tests are perfomed by veterinary doctors licensed by Ministério da Agricultura e Pecuária (MAPA) and in event of a positive or inconclusive result the veterinarian can administer the confirmatory test, the comparative cervical tuberculin test (CCT). It is mandatory for licensed veterinarians to report all tests results in Sistema de Gestão de Defesa Animal e Vegetal (GEDAVE), an online system administered by Coordenadoria de Defesa Agropecuária. All the data used for this analysis was extrated from GEDAVE.

Results

In 2020, all 3 bovines with positive results in caudal fold test (CFT) were negative in CCT; from 50 positive and 19 inconclusive results in single cervical test (SCT), 35 were positive in CCT and 25 negative. The 9 remaining animals were either stolen or died of non tuberculosis related causes. In addition, from 145 inconclusive results in CCT, 11 ended up positive, 126 negative and the remaining animals were either stolen or died of non tuberculosis related causes. The concordance values were 0% for CFT, 50,72% for SCT and 7,59% for inconclusive CCT. In 2021, 2 bovines with positive results in CFT were negative in CCT, while 1 was positive; from 13 positive and 50 inconclusive results in SCT, 6 were positive in CCT, 46 negative and 1 died of non tuberculosis related causes. From 162 inconclusive results in CCT, 48 ended up positive, 114 negative. The concordance values in 2021 were 33,33% for CFT, 11,32% for SCT and 29,63% for inconclusive CCT. In 2022, 9 bovines with positive results in CFT were submitted to CCT, resulting in 3 positive and 6 negative results; from 1 positive and 5 inconclusive results in SCT, 2 were positive in CCT and 4 were negative. From 22 inconclusive results in CCT, 7 ended up positive and 15 negative. The concordance values in 2022 were 33,33% for CFT along with SCT and 31,82% for inconclusive CCT.

Conclusions

The concordance values calculated demonstrate the confirmatory test is essential in order to avoid destroying false positives in routine tests.

1265 - Associations of Walking and Waiting Times with Milk Yield

Author: Paola Bacigalupo Sanguesa, Pamela Ruegg, Roger Thomson, Bo Norby, Ronald Erskine

Objectives

The objective of this study was to determine the associations between time spent walking from housing pens to the holding pen and waiting time in the holding pen and milk yield as the outcome variable.

Material and methods

We randomly selected 64 Holstein cows from a commercial dairy farm equipped with parlor software (DeLaval DelPro Software) that collects milking data. Cows were milked 3 times a day in a rotary parlor. The inclusion criteria were as follows: being housed in one of two selected group pens; being between 22 and 365 days in milk (DIM); no cases of clinical mastitis, lameness, or other health events during the lactation at enrollment; 4 functional quarters; lactation 1 through 5; no scheduled reproduction management during the study period; and daily milk yield within two standard deviations of the herd average. The enrolled cows were assigned and marked with a colored symbol in the rump area. We installed time lapse cameras (Brinno TLC300) at the exit of the housing pen and at the entrance of the holding pen to record images every 2 sec during 13 consecutive milkings. The time stamp of the videos was used to obtain the housing pen exit time and holding pen entrance time of the selected cows. We collected milking performance data (yield, milking session, milking start time) from the milking recording system software. Walking time (s) was calculated by subtracting housing pen exit time from holding pen entrance time. Time at holding pen (s) was calculated as the time between holding pen entrance time and milking start time. We performed multiple variable regression with repeated measures, including first order interaction terms, to explore the association between yield and the explanatory variables. Yield was analyzed as a continuous variable, as were walking time and time in the holding pen. Milking session (1,2,3), lactation (1st, 2nd, 3rd-5th), and DIM (021-100, 101-200, 201-365) were treated as categorical explanatory variables. Model selection was done by manual backward elimination. Microsoft Excel and SAS 9.4 were used for data management and statistical analysis.

Results

A total of 50 cows were part of the sample. The means for lactation, DIM, milk yield per session, walking time and time at the holding pen were 2.2, 193.1, 31.7, 3:14 minutes (134 s) and 67:58 minutes (40778 s), respectively. When cows were categorized by lactation, mean walking time increased (164 s, 192 s, 229 s for 1st, 2nd, and 3rd+ lactation, respectively) and mean holding pen time decreased as parity increased (4376 s, 41010 s, 3734 s for 1st, 2nd, and 3rd+ lactation, respectively). No trends were observed for DIM. The final model included time at holding pen, milking session, lactation, DIM, and the interaction between milking session and DIM. The model showed a positive association on yield as time at holding increased ($P < 0.001$).

Conclusions

Although this is a preliminary analysis with an incomplete data set, the results suggest that relative to yield, walking time might not be as relevant as the time spent waiting in the holding pen.

1326 - Effect of treatment with meloxicam on behavior and pain indicators in cows with clinical and subclinical mastitis in Ecuador

Author: Christian Albuja, Joel Hernández Cerón, Ramiro Rearte, Rodolfo Luzbel de la Sota

Objectives

To study the effect of treatment with meloxicam on behavior and pain indicators in cows with clinical and subclinical mastitis in Ecuador

Material and methods

Fifty crossbred Holstein cows from a commercial herd in Pichincha province, Ecuador, were used. The study began in December 2020 and ended in December 2021. A California Mastitis Test (CMT) was performed every two weeks on all cows with 2 to 5 lactations, open or inseminated, between 35 and 160 days in milk. All animals that presented a CMT score of 0 (CON), 2 (CMT2) and 3 or clinical (CMT3+C) were included in the study and randomly assigned into five groups; CON (healthy control cows, n=10), CMT2C (subclinical mastitis 2 without meloxicam, n=9), CMT2M (subclinical mastitis 2 with meloxicam, n=9), CMT3+CC (clinical and subclinical mastitis 3 without meloxicam, n=9) =11) and CMT3+CM (clinical and subclinical mastitis 3 with meloxicam, n=10). In the cows in the treated groups, meloxicam was used at a dose of 0.5 mg/kg (Metacam® Boehringer Ingelheim [2.5 ml/100 kg]) administered once subcutaneously. Cows with subclinical 3 and clinical mastitis were treated with an intramammary antibiotic for four milkings (tetracycline 200 mg, neomycin 250 mg, bacitracin 2000 IU, prednisolone 10 mg, Mastijet Fort®, MSD Salud Animal, Mexico). Cows with severe clinical mastitis with signs of toxemia were excluded from the study. To elucidate the impact of clinical and subclinical mastitis on animal welfare, measurements of pain indicators (body temperature [TEMP], rumen movements [RUMMOV], haptoglobin [HAPTO], serum amyloid A [AMIL], cortisol [CORT], somatic cell count [SCC], and behavior (general fitness [GENAPT], response to approach [RESPON], head position [HEADPOS] and ears [EARPOS], standing posture [STANPOS], appetite [APET]and facial expression [FACIAL). All these indicators were monitored and observed on days 0, 7, and 14 after the initial diagnosis. All response variables were analyzed by fitting a logistic regression model with group, treatment, and day as the main effects and its first and second order interactions (PROC GLIMMIX, SAS, 9.4).

Results

The TEMR did not show significant changes throughout the study due to the effect of group (P>0.22), day (P>0.08) and treatment with metacam (P>0.36), or their corresponding interactions (P>0.14). During the experiment, the concentrations of CORT (ng/ml), HAPTO (ug/ml), AMIL (ug/ml), CCS (x1000/ml), and RUMMOV (min) were different between the CON, CMT2, and CMT3+C (P<0.03). In all cases, the concentrations of CORT, HAPTO, AMIL, and SCC in the CON group were lower than in the CMT2 and CMT3+C groups (12.66±1.20 vs. 18.58±1.49, P=0.007; 4.31± 0.27 vs. 47.5±5.41, P=0.004; 0.35±0.03 vs. 0.56±0.05, P=0.001; 126.20±16.55 vs. 5070.35±803.91, P=0.0004).

On the contrary, RUMMOV was higher in the CON group than in the CMT2 and CMT3+C groups (2.60±0.1 vs. 1.93±0.1, P=0.001). Furthermore, the concentrations of HAPT, AMIL, and SCC were lower in the CMT2 group than in the CMT3+C group (16.68±3.75 vs.78.32±7.08, P<0.0001; 0.35±0.03 vs. 0.77±0.07, P= 0.001; 3287.29±757.54 vs. 6853.42±850.29, P=0.02; respectively).
Also, during the experiment, CORT and HAPTO concentrations differed between days 0, 7, and 14 (P<0.03). Only CORT and HAPTO concentrations were higher on day 0 than on days 7 and 14 (19.34±1.52 vs. 14.43±1.57, P=0.01; 50.021±8.75 vs. 35.84±6.43, P=0.003). Likewise, only HAPTO concentrations were higher on day 7 than on day 14 (42.76±7.91 vs. 28.093 vs. 4.95, P=0.02). Likewise, CORT, HAPTO, AMIL, and SCC concentrations decreased more markedly between days 0, 7, and 14 in cows treated with metacam compared to those not treated with metacam (interaction of metacam by day, P< 0.05).
Regarding behavioral changes, in the CMT2 group, there were no differences between the groups with and without metacam for the HEADPOS, EARPOS, or APET indicators; however, a favorable response was found in GENAPT (p=0.04), RESPTA (p=0.06), POSTP (p=0.03) and FACIAL (0.003). Similarly, in the CMT3+C group, there was no difference in GENAPT, EARPOS, or APET, while cows treated with meloxicam showed an improvement for RESPON (p=0.004), HEADPOS (p=0.01), STANPOS (0.02) and FACIAL (0.01).

Conclusions

It was evident that cows with clinical and subclinical mastitis had an increase in pain, inflammation, and behavior indicators. The cows treated with meloxicam showed a decrease in these indicators, suggesting an excellent therapeutic alternative to improve animal well-being in cows with clinical and subclinical mastitis.

1333 - Tail tip lesions in dairy cows – a look on the inside

Author: Lea M. Lorenz, Marielle Volkwein, Christine Schmidt, Mirjam Lechner, Prisca V. Kremer-Rücker

Objectives

In our previous work, we observed a variety of tail tip lesions in dairy cows, which we classified into 7 different clusters. Prevalences of severe lesions such as bleeding or necrotic tail tips reached up to 26 %. These results might indicate that we are dealing with an animal health and welfare issue that has gone unnoticed by now. In fattening cattle, histopathological examination of affected tails led to the presumption that the observed lesions might be caused by cutaneous ischemia. The objective of this study was therefore to find out which histopathological changes underlie the different tail tip lesions in dairy cows in order to contribute to a better understanding of this malady.

Material and methods

We collected 16 tails of dairy cows of different breeds (Holstein Friesian, Simmental, Brown Swiss and crossbreeds between beef- and dairy breeds) at a slaughterhouse in South Germany after slaughtering. The tail tips were selected to cover all types of macroscopically visible lesions that we have observed in previous studies. Selected samples originated from cows aged two to ten years. After collecting, the tails were packed on ice during the one hour transport. Immediately after arrival, tail tips were shorn, washed, photo documented and examined macroscopically. For histopathological examination, observed lesions were fixed in 10 % buffered formalin for at least four days. Subsequently, all samples were embedded in paraffin, processed routinely for histologic examination, and stained with hematoxylin and eosin (H&E). Selected sections were also stained with periodic acid Schiff reaction (PAS) or Masson-Goldner trichrome for examination of connective tissue. The slides were examined using light microscopy.

Results

In all cases of macroscopically visible constrictions as well as in hairless areas of the tail tips we found dermal granulation tissue of different age stages that extended to the central longitudinally arranged connective tissue of the tail. In six out of ten tail tips with macroscopically visible annular constrictions, the epidermis was without any particular finding. In the other four cases, we found inflammation of the epidermis with underlying cell-rich granulation tissue. The epidermis of samples showing alopecic tail tips was either free from pathological findings or showed signs of orthokeratotic hyperkeratosis. In tail tips macroscpically exhibiting hyperkeratosis, we observed predominantly orthokeratotic and partly also parakeratotic hyperkeratosis. In one macroscopically unremarkable tail, histological examination also revealed hyperkeratosis. Tail tips with macroscopically visible swelling of the central longitudinal connective tissue were histologically characterized by extracellular and low protein edema. Intracellular edema could be observed in the marginal areas of constrictions with bloody encrustations and in hairless areas. In tail tips which showed macroscopically visible thinning, all layers of the tail were reduced in thickness. Only in one tail tip, we found a concrete thinning of the dermis on only one side of the tail distal to a constriction. Another frequent histopathological finding was the partly focal, partly diffuse hyperemia of the papillary bodies in the dermis, which was found in ten samples. We observed hyperemia in areas

affected by hyperkeratosis and in annular constrictions, but also in macroscopically unconspicuous tail tips, in hairless areas and in tail tips showing hyperkeratosis or necrosis. In some cases, the hyperemia was accompanied by free erythrocytes, whereas no accumulation of inflammatory cells could be detected in these areas.

Conclusions

Our histopathological examination of tail tip lesions in dairy cows revealed amongst others granulation tissue formation, ortho- and parakeratotic hyperkeratosis and hyperemia of the papillary bodies with free erythrocytes. These results resemble those found in fattening cattle. Based on the macroscopical and histological appearance of the lesions, trauma as the sole cause seems unlikely. Future research is required to clarify the underlying etiology and to determine risk factors associated with the observed lesions.

Funding

Parts of this research were funded by the Tönnies Forschung, Rheda, Germany.

1334 - First insights into possible risk factors for tail tip lesions in dairy cows

Author: Prisca V. Kremer-Rücker, Lea M. Lorenz, Kim F. Schubert, Saskia Meier, Mirjam Lechner

Objectives
Although inflammation and necrosis of the tail tip has been a well-known problem in fattening cattle for several decades, the etiology of the disease is not fully understood, yet. While some authors hold husbandry conditions such as limited space allotment or slatted concrete floors responsible for the occurrence of tail lesions, other studies point towards a connection with rumen acidosis. Furthermore, tail necrosis has also been described in the context of mycotoxin intake, which leads to vasoconstriction, finally resulting in ischemia. In dairy cows, we observed lesions of the tail, which resemble those described in housed fattening cattle, although husbandry conditions of dairy cows differ from those of fattening cattle. In order to identify possible risk factors, the aim of this study was to investigate whether there are associations between the occurrence of tail tip lesions and selected health and performance parameters in dairy cows.
Material and methods
For this purpose, we monitored the course of the lesions over 10 months in a German dairy herd consistig of 1,300 cows. The cows were housed on slatted rubber floor in a loose housing system. The cubicles either contained straw manure bedding or were covered with waterbed mattresses. Manure was removed by an automatic scraper system. N = 213 cows that calved within a predetermined period of 2 months were selected for the study to ensure that all cows were at a similar stage of lactation. Number of lactation varied between cows, ranging from one to seven. The sample consisted of 89% Holstein Friesian cows, 10% crossbreds and 1% Brown Swiss cows. Examinations took place at every milk performance testing, starting after calving. One single person assessed tail tip lesions, locomotion score and body condition score of the cows. Number of lactation, days in milk, and data from milk performance testing including milk yield, milk fat:protein ratio and somatic cell count were used for further analysis. The frequencies of the individual tail lesions were calculated by dividing the number of each lesion by the number of all observed lesions. Data were processed using Rstudio version 2021.09.2. Chi^2-test and a mixed model procedure were performed for statistical analysis of possible risk factors for the different lesions.
Results
Over the course of the examination period, every cow was affected by one or several lesions at least once. The examination of n = 213 cows resulted in 4,123 observations. We were able to observe all of the previously described lesions, with varying frequencies, namely: lesions at the very tip of the tail: 8.2 %, annular lesions: 8.5 %, hyperkeratosis: 52.0 %, swelling: 10.7 %, axis deviation: 9.2 %, thinning: 0.2 % and fibroma: 3.7 %. The frequency of tails without any visible lesion was 7.5 %. Regarding the results of the Chi^2-test, with the exception of the somatic cell count, all parameters included in this study had a significant influence on at least one lesion. Days in milk significantly influenced all lesions ($p < 0.01$ or $p < 0.001$) except of thinnings of the tail. Lactation number had a significant impact on the occurrence of hyperkeratosis ($p < 0.05$), swellings ($p < 0.001$), axis deviations ($p < 0.01$) and fibromas ($p < 0.001$). Hyperkeratosis and axis deviations were furthermore influenced by milk yield ($p < 0.05$

and $p < 0.001$, respectively). Milk fat:protein ratio had a significant impact on hyperkeratosis ($p < 0.05$) and thinning ($p < 0.001$). Locomotion score significantly influenced annular lesions ($p < 0.05$) and axis deviations ($p < 0.05$), whereas body condition score had a significant impact on the occurrence of swellings ($p < 0.001$).

Conclusions

Our results demonstrate that there are multiple associations between tail tip lesions and different health- and performance parameters such as milk yield, locomotion score and body condition score. Based on our results, we conclude that the formation and development of tail tip lesions is a complex, presumably multi-factorial process. Further research is required to examine the individual risk factors in more detail.

Funding

Parts of this research were funded by the Tönnies Forschung, Rheda, Germany.

1369 - Animal and feeding behavior and energy metabolism of Holstein dairy cows in the transition period receiving Bovine Appeasing Substance

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Objectives
The objective of this study was to evaluate the effects of the use of Bovine Appeasing Substance (BAS) on the feeding and animal behavior and energy metabolism of Holstein dairy cows in the transition period.
Material and methods
Twenty-four multiparous cows were distributed into two groups, in a completely randomized design: Control Group (n=12, without BAS application) and BAS Group (n=12, with product application). The animals were followed up between 28 days prepartum and 21 days postpartum. The application of BAS (SecureCattle®-Nutricorp©, Campinas-Brazil) was carried out on days -28, -14 and 0 in relation to parturition, at a dose of 5ml topically, on the nape of the neck. The groups remained at a minimum distance of 50 meters throughout the evaluated period to avoid influence of the product on the Control group, given the volatility of the product. The animals remained in a confined Compost barn system, receiving totally mixed ratio twice a day formulated in accordance with NRC (2021), water *ad libitum* and were milked during the morning and afternoon. Milk yield data were obtained directly from the milking system for the software DelPro™ (DeLaval©, Jaguariúna, Brazil). Smart feeders (Intergado®, Betim, Brazil) were used to assess consumption and eating behavior automatically and individually, with intake of natural matter being subsequently converted into intake of dry matter (DMI). Animal monitoring collars CowMed© (ChipInside®, Santa Maria, Brazil) were used to assess rumination, activity, idleness and panting. The milk efficiency of the groups was calculated by dividing milk production by DMI. The live weight of the animals was obtained using a weighing tape and the body condition score was evaluated from 1 to 5, on a scale of 0.25 points. Blood samples were collected on days 28 and 14 pre-partum and 0, 7, 14 and 21 post-partum through the coccygeal vein of the tail for analysis non-esterified fatty acids (NEFA), betahydroxybutyrate (BHB) and glucose. The data obtained were analyzed using the statistical software JMP Pro 14 and the means were analyzed using the MIXED MODEL procedure, considering the animal, the treatment, the time of collection and their interactions. Values of $p < 0.05$ were considered significant.
Results
The animals in the BAS group had lower DMI (-3,97 kg prepartum and -3,33 kg postpartum) ($p < 0.05$), higher milk yield (+2.92 L of milk ($p < 0.05$), higher milk efficiency ($p < 0.05$), with greater postpartum rumination time ($p < 0.03$), longer activity time ($p < 0.05$) and less postpartum idleness ($p < 0.05$) in relation to the Control group. The BAS group also had lower numbers of views of feeders, total time and time consumed than the Control group ($p < 0.05$). No differences were found in body weight ($p > 0.05$) and BCS ($p > 0.05$) of the animals, as well as in postpartum rumination time ($p > 0.05$). No differences were found in NEFA, BHB and glucose between the groups ($p > 0.05$).
Conclusions

The animals treated with BAS showed greater milk production and lower dry matter intake, with the greater postpartum rumination time than the Control group in transition period.

1382 - Temperature asymmetries as indicators of emotional valence: Calves' behavioural responses to the camera.

Author: Marco Antonio Ramirez Montes de Oca, Mike Mendl, Helena telkänranta, Suzanne Held, Sarah Lambton

Objectives

Emotions are short-term affective states that can be assessed in terms of valence (positive or negative) and arousal (low or high). Some of the most common ways used to assess the affective states in animals require specialized training (like cognitive tasks) or involve the restraining or attachment of equipment to the individuals (HR, HR variability, EEG or fMRI) which can stress the animals and elicit an emotional response per se. For this reason, the development of objective non-invasive techniques for the study of animal emotions is required. So far, infrared thermography has been used successfully to identify arousal levels in animals due to changes in the sympathovagal balance. However, the interpretation of those changes in terms of emotional valence remains unclear.

For these reasons, we decided to explore the hypothesis that temperature asymmetries could arise from a previously studied characteristic of the brain: emotions are processed differently in both hemispheres of the brain, with emotions resulting in behavioural approach (generally more positive emotions) processed more intensely on the left hemisphere, whereas emotions resulting in behavioural withdrawal (negative emotions) processed more intensely in the right hemisphere. We hypothesise that temperature asymmetries (difference in temperatures between left and right sides of bilateral areas, i.e. eyes, nostrils or ears) measured with infrared thermography could reflect higher activity of one of the brain hemispheres due to increased blood flow or muscular activity in the area. For this purpose, we recorded temperature changes in different regions of interest (ROIs) in the head of dairy calves and scored the behavioural responses of the calves to the researcher during the recording.

Material and methods

Thirty-six female Holstein Friesian calves aged between 20 and 111 days from the University of Bristol's dairy farm were selected for the study. The calves were kept in groups of 6 to 8 calves and feed twice a day at 9 am and 4 pm. The calves were divided into three groups for a separate experiment looking at emotional changes in response to being disbudded and observing conspecifics being disbudded with a hot iron. For comparing behavioural responses, 4 recordings were used: baselines recorded at 1 pm and 5 pm on the day before any intervention and at the same times on the day after the intervention. Thermal video of each calf was recorded for 5 minutes, and from this thermal video, the experimenter collected at least one thermal image in perfect focus from each of 4 directions with regard to the calf's head (left, right, front and back). During each recording, the number of times that the animal moved towards and away from the researcher was counted and scored as Approaching (more moves towards than away from the researcher), Moving away (more moves away than towards the researcher) or staying still if no movement was recorded. Simple regression models for the different regions of interest (inner corners of the eyes, nostrils, back of the ears and front of the ears) were carried out, testing for the effects of calf age, ambient temperature, time of recording and response to the camera/researcher.

Results

Asymmetries in maximum temperature of the inner corner of the eye were associated with the response of the calves to the camera ($\chi 2$ =7.09, df=2, p=0.028), with calves approaching the camera having significantly warmer right eyes (suggesting left brain hemisphere activity) compared to the calves that moved away from the researcher showing higher left eye temperatures (p=0.007). Calves staying still did not differ significantly from the other groups. There were no significant differences associated with the variables of interest (response to the camera) for the ear maximum temperature asymmetries (front and back view) or in temperature asymmetries in the nostril areas.

Conclusions

Temperature asymmetries in the inner corner of the eye measured by infrared thermography were correlated with approach/withdrawal behaviour, and hence calves' assumed emotional valence, suggesting potential for developing novel thermographic methodologies of animal affective states. However, as this research is at an early stage, caution must be taken when interpreting results. Further work is needed to validate these findings, and to determine whether there are other factors besides valence such as processing of visual and auditory stimuli in different hemisphere of the brain that can cause similar temperature effects during calf responses to people.

1398 - Assessment Welfare protocol to a buffalo farm

Author: Melina Yasuoka Marie

Objectives

This summary aims to contribute expertise in the welfare evaluation of buffalos, and adept at conducting comprehensive assessments and implementing welfare enhancement strategies.

Material and methods

There were evaluated 1160 Murrah buffalos on a farm located at Brotas, São Paulo, Brazil. The body condition score (BCS) assessment was identified with the animals brought from lots of farm paddocks, inspected one by one, contained 4-5 animals in the trunk hose/trough in single file, individually marked with a stick and paint, photographed and counted, and at the end of each batch, the animals returned to their paddock to start the next batch. The BCS methodology used was according to Alapati and collaborators (2010), Welfare Quality (2009) and De Rosa et al.(2007).

The animals evaluated were separated in 6 lots (males, before calving, mixed lot, new maternity (calves 30-90 days), old maternity (calves at weaning to pubertal), and after calving). The feeding points and environment were evaluated by inspecting the feeders and drinker, paddocks, and indicated by current managers. And health and behavior assessment were identified with the animals brought from lots of farm paddocks, inspected one by one, contained 4-5 animals in the trunk hose/breech in single file, individually marked with crayon stick, photographed and counted, and at the end of each batch the animals returned to their paddock to start the next batch.

Results

The protocol proposes the final diagnosis of well-being on a scale of five degrees: very high, high, regular, low and very low, using a simplified form of integration. The final conclusion for a very low level of well-being will be defined when three or more sets of indicators are classified as inadequate or when there is intentional physical aggression; a low level of well-being will be determined when there is an inadequacy of one or two sets of indicators; regular level of well-being when two or more sets of indicators are considered regular and none is considered inadequate, high level of well-being when only one set of indicators is regular and very high level of well-being when all sets are adequate of indicators (DE ROSA,et al., 2007).

Was observed that all groups with the exception of the “after calving” lot, the others groups have a satisfactory level of well-being on the farm, the batch of recently calved animals had low body scores (BCS 1 and 2), the feeders were full of food and water, but as long as the animals have this low score, the diet needs to be adjusted to meet the animal's demands during this period. Drying very thin cows is of great importance as it reduces one of the energy demand pathways. Carry out artificial feeding to ensure the health of calves with dry mothers. The environment was extremely humid, without any grass, disqualifying the place for postpartum cows and newborn calves, considering that their immunity is low.

Conclusions

Assessing buffalo welfare involves considering various factors, including their physical health, behavioral needs, living conditions, and the overall management practices. Here

are some key aspects to consider in buffalo welfare evaluation: 1.Ensure that buffalos receive a balanced and adequate diet to their nutritional requirements. Access to clean water and appropriate feed is crucial; 2.Evaluate the living conditions of buffalos, including the availability of shelter and protection from extreme weather conditions. Adequate space, proper ventilation, and comfortable resting areas are essential; 3. Regular veterinary care for maintaining buffalo health. Monitoring and addressing any signs of illness promptly is essential. Vaccinations and disease prevention measures should be in place; 4.Buffalos has natural behaviors that need to be considered. They should have enough space to exhibit natural behaviors such as grazing, socializing, and resting. Enrichment activities can also contribute to their well-being; 5.If buffalos are in reproduction programmer, it's important to ensure that breeding practices are ethical and considerate of the animals' well-being. Monitoring the health of calves and providing appropriate care is essential; 6.Buffalos are social animals, and their well-being is influenced by social interactions. Ensuring that they are kept in compatible groups and minimizing social stress is important (BROOM & MOLENTO, 2004; DE ROSA, ,et al., 2005, 2007; WELFARE QUALITY, 2009; AMARAL, et al., 2019).

1400 - USO DE LOS BEBEDEROS EN HEMBRAS DE LA RAZA DE LIDIA. RELACIÓN CON EL BIENESTAR ANIMAL EN EXTENSIVO.

Author: JUAN MANUEL LOMILLOS PÉREZ, Pablo Iglesias Santiago, Marta Alonso

Objectives

El agua es un nutriente básico e indispensable para la vida (Wagner y Engle, 2021). El consumo de una cantidad adecuada es esencial tanto para la salud y bienestar animal como para la producción, pues en condiciones normales, un bovino adulto pierde un 6.5% de su peso vivo diariamente, debido la respiración, la orina y las defecaciones. Esta cantidad debe ser recuperada mediante la ingesta directa de agua de bebida y, en menor medida, mediante la ingestión de forrajes ricos en agua.

Tradicionalmente se ha considerado que el patrón de bebida era un factor de especial trascendencia en la producción láctea, por lo que la mayoría de los estudios realizados hasta la fecha se han llevado a cabo sobre animales lecheros. No obstante, teniendo en cuenta su influencia sobre la ingestión de alimentos sólidos y la correlación con la ganancia de peso y condición corporal (Meyer et al., 2006, Brew et al., 2011) nos planteamos la necesidad de llevar a cabo este estudio en extensivo y en concreto en la raza de Lidia.

Por otro lado, los efectos del cambio climático sobre la disponibilidad de agua y el uso que se hace en la producción ganadera de los recursos hídricos son un motivo de creciente preocupación social incrementando la presión para que se adopten prácticas agrícolas sostenibles y eficientes (Pires et al., 2022) sin que afecten al bienestar animal.

En ganado vacuno de carne se ha comprobado que existe una correlación positiva entre la ingesta de agua y la ganancia diaria de peso (Ahlberg et al., 2018 y Brew et al., 2011) y el consumo de materia seca (Meyer et al., 2006 y Brew et al., 2011). Los valores de referencia oscilan entre los 2,3 y 4 litros de agua por cada kg de materia seca (Meyer et al., 2006), existiendo una bajada del consumo de alimento entre 5 y un 23% cuando se producen limitaciones en el acceso al agua del 60 al 80%.

Aunque desde el punto de vista productivo todo el manejo realizado en las ganaderías de ganado bravo influirá en los rendimientos finales (Lomillos et al., 2012) existen algunos momentos críticos, como el comprendido entre el destete y la tienta de las hembras, siendo necesario que las añojas tengan, durante esta época, un correcto crecimiento para llegar al momento de la tienta con un buen desarrollo y condición corporal que les permitan soportar el proceso de tienta y encontrarse en plenas facultades para demostrar su valía como futuras reproductoras. Además, deben llegar en un estado óptimo para facilitar la preñez y llevar a término una gestación en el caso de que en el proceso de tienta fuesen seleccionadas como futuras madres.

El ritmo de ingesta de agua está correlacionado con el consumo de alimento y se lleva a cabo durante el periodo de alimentación o poco después de la misma (Cardot et al., 2008). Concretamente un 34.5% del total de agua consumida al día es ingerida en las 2 horas posteriores a los episodios pandriales estando los osmorreceptores ruminales involucrados en dicho proceso pues la infusión de agua en el rumen estimula la alimentación.

El objetivo del presente trabajo es evaluar la influencia de la suplementación alimentaria sobre el comportamiento social y el uso circadiano de los abrevaderos por parte de hembras de lidia, empleando la técnica del fototrampeo, muy útil en este tipo de

animales criados en sistemas extensivos de pastoreo libre, que generan respuestas de huida ante la presencia humana, pues permite obtener información de la presencia en un espacio físico y del comportamiento de animales que viven en grandes extensiones sin interferir en su etología. La información obtenida permitirá diseñar estrategias de manejo más adecuadas para minimizar el impacto sobre el bienestar y la producción de la escasez de agua debido al escenario actual de cambio climático con periodos prolongados de elevadas temperaturas y menores precipitaciones.

Material and methods

Animales utilizados y Área de estudio

En este estudio se emplearon 20 hembras entre uno y medio y dos años de la raza bovina de Lidia.

La realización del estudio se llevó a cabo en la finca adehesada de la Ganadería de Valdellán, situada entre los municipios de Santa María del Río, Villamizar y Villacalabuey, en la provincia de León (42º 31' 06.5" latitud N y 6º 17' 5" longitud W). Está situada en una zona de monte bajo entre las riberas de los ríos Cea y Esla y tiene una extensión aproximada de unas 500 hectáreas.

Los cercados empleados en el estudio fueron los siguientes:

- Foncabada: en él se realizó la primera parte del estudio. Extensión 12 hectáreas.
- La Caza: en él se realizó la segunda parte del estudio. Extensión 26 hectáreas.

La finca combina amplias praderas y robles adehesados, que sirven de abrigo y refugio natural a las reses que pastan libremente en diferentes cercados, a lo que se suman sus condiciones orográficas con zonas planas y laderas pronunciadas que permiten tanto el ejercicio de los animales como su reposo. Existe abundancia de agua en forma de arroyos y manantiales que riegan y abastecen de norte a sur toda su extensión, y tan solo los cercados objeto de estudio no disponen de ninguna fuente natural de agua.

Bebederos utilizados

- Foncabada: forma rectangular con 2,45 metros de longitud por 1 metro de ancho y 0,5 metros de profundidad. 690 cm de acceso lineal por cuatro lados.
- La Caza: forma rectangular 5,6 metros de largo por 2,5 metros de ancho y 0,5 metros de profundidad. 820 cm de acceso lineal por tres lados.

Ambos son el único punto de agua y están construidos con hormigón y el mecanismo de funcionamiento consiste en una boya que regula el caudal y asegura un nivel constante de agua. El agua procede de un sondeo que también abastece la casa y resto de dependencias: plaza de tientas, corrales y cuadras.

Manejo de la alimentación durante el periodo de estudio

En el primer período, llevado a cabo en el cercado de Foncabada durante el mes de septiembre, debido a la climatología típica de la estación estival precedente, los recursos herbáceos eran muy escasos. Por ello, fue necesario suplementar la alimentación de las añojas con 2 kg de concentrado diarios por animal, los cuales se repartían en dos momentos, entre las 9 y las 10 horas y entre las 15 y las 16 horas, 1 kg en cada toma.

En el segundo período, desarrollado durante el mes de diciembre, debido a que la estación otoñal precedente había sido muy buena climatológicamente y permitió un adecuado crecimiento herbáceo, se decidió el traslado de los animales desde el cercado de Foncabada al cercado de La Caza, donde las añojas se alimentaban únicamente a base de la hierba que ingerían mediante pastoreo. El motivo de este traslado fue aprovechar los recursos herbáceos de los diferentes cercados, buscando lograr un ahorro en el

capítulo de alimentación que siempre es el más costoso en la producción de animales para reposición.

Cámaras de fototrampeo

Las cámaras digitales utilizadas corresponden al modelo 119935 TRAIL SCOUT BUSHNELL® con sensores de movimiento IR utilizándose durante todo el periodo de estudio una en cada localización, aunque anteriormente habían sido colocadas dos cámaras con el fin de contrastar datos y ver que no se producían errores por mal funcionamiento de las cámaras. Se fijaron en robles en frente de cada bebedero a una distancia aproximada de 8 metros de los bebederos.

Las cámaras fueron programadas para funcionar de forma continua (24 horas por día), con toma de imágenes automática al activarse el sensor de movimiento IR con un intervalo de cuarenta segundos entre fotografías sucesivas, realizando vídeos de 5 segundos de duración, que se almacenaron en las tarjetas de memoria de 4 G.

Análisis de las fotografías y procesado de los datos

Las imágenes obtenidas a lo largo del estudio fueron almacenadas en un disco duro. Se obtuvieron un total de 1708 videos de 5 segundos de duración en los meses de septiembre y diciembre. Para realizar el análisis de los datos fue necesario procesar la información obtenida en forma de fotografías, una por cada vídeo de 5 segundos, y transformarla en datos numéricos. Este trabajo se realizó a través de la observación visual e interpretación de los animales presentes en las fotografías, así como la fecha y hora de las mismas. Las fotografías fueron tratadas como eventos individualizados debido a que no se podía tener la certeza de hacer un seguimiento individual de cada animal.

La visualización de las fotografías se llevó a cabo mediante el programa informático Lenovo Photo Master®, recogiéndose los datos numéricos en una hoja de cálculo del programa Microsoft Excel® para WINDOWS® creada para tal efecto, siendo procesados mediante hojas dinámicas.

Results

En los Gráficos 1 y 2 se presentan los datos de las añojas que fueron fotografiadas bebiendo o en el entorno próximo al bebedero, distribuidas por horas, durante los dos periodos de estudio. La existencia de un patrón circadiano con momentos de mayor utilización de los bebederos resulta evidente en ambos Gráficos, alcanzándose el valor máximo de animales bebiendo a las 11 y a las 18 horas en el caso del primer periodo con suplementación de concentrado en el cercado de Foncabada (Gráfico 1) y a las 10 horas en el segundo periodo, sin suplementación, en el cercado de La Caza (Gráfico 29.

De este modo, nuestros resultados concuerdan con diferentes estudios realizados en sistemas intensivos sobre las horas de mayor utilización de los bebederos, en los cuales se observaba que la mayor parte del agua ingerida a lo largo del día era consumida pocas horas después de la ingesta de alimento. Los episodios de bebida tuvieron lugar en las dos horas posteriores a los periodos de administración de concentrado demostrando la relación existente entre la ingesta de alimento (concentrado) y de agua, pues la presencia de concentrado en el rumen incrementa la osmolaridad del contenido ruminal, se genera deshidratación (Scott et al., 2011), lo que incita al consumo de agua para disminuir dicha osmolaridad. Por ello, resulta de gran importancia que los animales dispongan de agua después de la toma de la mañana para que no sea factor limitante de la ingestión de más alimento concentrado en la siguiente administración.

Nuestros resultados están de acuerdo con los estudios de Osborne et al. (2002) pues no constatamos un menor uso del bebedero cuando temperatura del agua era muy baja en los meses más fríos, ni que hicieran un uso más frecuente de éste para refrescarse en septiembre, comportamiento que si había sido comprobado en ganado vacuno de otras razas (Rouda et al., 1994). A pesar de la ingestión de concentrado cuyo metabolismo podrían incrementar el efecto del calor ambiental durante el mes de septiembre, no precisan una elevada ingestión de agua para contrarrestar el calor, posiblemente debido a que es una raza adaptada a las elevadas temperaturas, lo que no ocurre con otras razas con menor rusticidad (Beaver et al, 1989).

Comprobamos que el ritmo circadiano de ingestión de agua se vio influido en gran medida por la suplementación con concentrado dado que en el periodo de diciembre (Gráfico 2) observamos un único pico de bebida durante el período matinal, lo cual concuerda, en parte, con los resultados obtenidos por Rossi et al, en 1999, donde indican que bajo condiciones de alimentación *ad libitum*, los picos de ingesta de alimentos y de agua se producen en la primera hora de la fase de luz y la primera hora de la fase oscura. En nuestro estudio observamos que se produce el pico de ingesta de agua en las primeras horas de fase de luz, pero en cambio no se observa ningún acontecimiento de ingesta de agua al inicio de la fase oscura. Este resultado concuerda con la afirmación de Rouda et al. (1994) de que la mayoría de las vacas solo necesitan una única ingestión de agua al día. Como se puede observar en el Gráfico 2, durante el pico de mayor ingesta de agua, vemos que hay una diferencia excesiva entre los animales que beben y los animales registrados en la zona. Esto es debido a la presencia de hielo en el bebedero en las primeras horas de la mañana en el mes de diciembre, lo cual limita de forma muy importante el acceso al agua, y dado que los animales se rigen en este caso principalmente por su ritmo circadiano más que por las características del agua, sería muy recomendable realizar todas las mañanas una revisión de bebederos para retirar el hielo. La presencia de hielo alarga considerablemente el tiempo de espera de los animales alrededor del bebedero, y como podemos observar en el Gráfico 4, ese pico de ingesta coincide con el pico de máximas interacciones agonísticas, debido a que son los animales dominantes los que primero desean acceder al agua.

Por otro lado, nuestros resultados coinciden con Lardner et al. (2013) quienes afirman que en sistemas de pastoreo los grupos de animales tienden a acudir juntos a las fuentes administradoras de agua (Gráficos 1 y 2). A pesar de que el tiempo de permanencia en el entorno de los bebederos es breve, se produce una concentración de animales que incide en la aparición de patrones agonísticos (Gráficos 3 y 4), pues hay un número relativamente elevado de animales para el tamaño de los bebederos, menor en el caso del cercado de Foncabada, pero que siempre supera las dimensiones recomendadas para ganado vacuno. El tiempo de espera para poder acceder al bebedero repercute en una reducción del tiempo disponible para la ingesta de alimento y descanso. Paralelamente, la limitación en el acceso a este recurso genera peleas y golpes, con el consiguiente estrés de los animales, lo que se traduce en empeoramiento de rendimientos y mayor riesgo de pérdida de animales por lesiones, como indicaron en sus estudios Phillips y Rind, (2002). En el caso de las vacas objeto de nuestro estudio podría afectar especialmente a los animales subordinados, tal y como observaron que sucedía en vacuno lechero McDonald et al. en 2020. La repercusión en el ganado bravo es aún mayor pues no solo se ve afectado su bienestar, sino que la reducción en el tiempo de ingesta de alimento podría disminuir el potencial de crecimiento y desarrollo,

impidiendo realizar el tentadero en condiciones óptimas, con la consiguiente pérdida de potencial genético para la ganadería (Lomillos et al. 2013).
El conocimiento del patrón de bebida y la identificación mediante este estudio de problemas, como el tamaño insuficiente del bebedero o la presencia de hielo, resulta de gran interés para que los ganaderos puedan establecer estrategias de manejo y realizar mejoras encaminadas a maximizar el rendimiento de sus animales.

Gráfico 1: Número total de hembras bebiendo y presentes en el entorno del bebedero durante las distintas horas en el periodo con suplementación de concentrado.

Gráfico 2: Número total de hembras bebiendo y presentes en el entorno del bebedero durante las distintas horas en el periodo sin suplementación de concentrado.

Gráfico 3: Patrones de comportamiento durante la ingesta de agua o en el entorno del bebedero durante las distintas horas en el periodo con suplementación de concentrado.

Gráfico 4: Patrones de comportamiento durante la ingesta de agua o en el entorno del bebedero durante las distintas horas en el periodo sin suplementación de concentrado.

Conclusions

13. Se comprueba la existencia de un patrón circadiano con intervalos marcados de utilización de los bebederos por parte de los animales de la raza bovina de Lidia estudiados, siendo ocasional el resto de episodios de bebida.
14. Se observa una clara relación entre los periodos de suplementación de la alimentación y los patrones de ingesta de agua, siendo notoria la influencia del aporte de concentrado.
15. Se detecta una permanencia de las vacas en el entorno del bebedero debido a un tamaño insuficiente de la fuente de agua y presencia de hielo, lo cual puede repercutir negativamente en su bienestar y rendimiento productivo.

1403 - Bienestar Animal en explotaciones de ganado bovino de lidia en España y México

Author: JUAN MANUEL LOMILLOS PÉREZ, Georgina Hernandez, Urso Davila

Objectives

Animales objeto de estudio. El presente trabajo se llevó a cabo en 6 ganaderías de ganado bovino de lidia que se encuentran en distintas localizaciones en la región de Extremadura (España) y en la región de Querétaro (México). Posteriormente, cuando se haga referencia a las ganaderías, se nombrarán como "Ganadería 1", "Ganadería 2", "Ganadería 3",respectivamente. Se evaluaron los animales dividiéndolos en grupos en función del tipo de producción, del sexo y a su vez, las vacas madres según los lotes en los que se encontraban divididas de la siguiente forma: sementales, vacas madres (lotes), añojos y añojas (animales de un año), erales y eralas (animales de dos años), utreros (machos de 3 años), cuatreños (machos de 4 años) y cinqueños (machos de 5 años) (TABLA I). En cada explotación se estudiaron 4 lotes de cada tipo de animal y 10 animales de cada lote. En aquellos lotes en los que el número total de animales fue menor de 10, se muestrearon todos los componentes del grupo.

Material and methods

La metodología de toma de datos desarrollada funcionó de forma adecuada. En general, las 6 ganaderías presentan una calificación óptima en todos los parámetros, si bien cabe destacar diferencias en cuanto a la distancia de aproximación, siendo menor en aquellas explotaciones en las que se manejan con mayor frecuencia a los animales, también reflejado en la curiosidad mostrada por los animales. Es importante destacar que el estudio refleja una alimentación adecuada, tanto a largo como a corto plazo (condición corporal y llenado ruminal) en las 6 ganaderías. En cuanto a la salud (respiración y mucosas, estado de pezuñas e índice de cojeras) se obtuvieron unos valores óptimos de BA. Se aprecia cierta diferencia entre las ganaderías de España y México en cuanto a la condición corporal de los animales, siendo sensiblemente menor en México,
donde en invierno es la época seca, con menor cantidad de pasto.

Results

El protocolo utilizado para la evaluación del BA en este estudio, podría ser empleado en cualquier explotación de ganado vacuno de lidia, ya que es rápido y sencillo de utilizar, además permite tomar los datos sin alterar los comportamientos naturales propios de la raza ni influir negativamente en los animales. Los parámetros relativos a comederos y bebederos muestran un buen estado de los mismos y diferencias entre explotaciones en los aspectos relativos a la presencia de agua o comida y su limpieza. Existen ciertos indicadores como son los índices de suciedad, de cojeras, estado de la ubre, estado de las pezuñas y de las mucosas, que presentan muy poca variación entre ganaderías e individuos del rebaño en el presente estudio, ya que los animales se encuentran en condiciones de semilibertad en amplios cercados, por lo que los valores de estos parámetros siempre son muy positivos con respecto al BA. Igualmente, los índices de acicalamiento, rumia, decúbito y comer, son muy dependientes del manejo alimentario de cada explotación y no registran variaciones concluyentes entre explotaciones ni entre tipos de animal es muestreados. Se detectan ciertas diferencias entre las calificaciones medias de los parámetros evaluados en las diferentes explotaciones, como es el caso de los indicadores de relación con el factor humano las prácticas de manejo, propias de

cada explotación y el mayor o menor contacto entre animal–humano. Ese es el caso de la distancia de huida, con diferencias entre ganaderías que manejan los animales más o menos. Se comprueba cómo, a pesar de su fuerte carácter y su marcado comportamiento agonístico, evidenciado en una mayor tasa de rozaduras, las señales de miedo ante la presencia del evaluador varían igualmente en función del tipo de animal, detectado diferencias entre los diferentes grupos de animales siendo los cuatreños y cinqueños los animales con más tasa de miedo y distancia de huida frente a vacas y sementales, más acostumbrados a la presencia humana. Los signos de curiosidad se manifiestan en mayor medida en los animales de un año.

Finalmente, considerando globalmente los valores obtenidos para los diferentes indicadores, se puede concluir que las 6 ganaderías estudiadas cumplen los requisitos de BA recomendados, encontrándose los animales en los momentos valorados en unas condiciones óptimas. A pesar de ello, se apreciaron diferencias entre las ganaderías de España y México en cuanto a la condición corporal de los lotes de animales jóvenes, que en el caso de México tienen menos nota, posiblemente por la escasez de pasto durante la estación invernal en las fincas dedicadas al ganado de lidia. Los cuatreños y cinqueños son los individuos que mayor condición corporal tienen en todas las ganaderías, por su manejo de alimentación intensivo, lo que queda igualmente reflejado en unas heces más blandas.

Conclusions

El protocolo utilizado para la evaluación del BA en este estudio, podría ser empleado en cualquier explotación de ganado vacuno de lidia, ya que es rápido y sencillo de utilizar, además permite tomar los datos sin alterar los comportamientos naturales propios de la raza ni influir negativamente en los animales. Los parámetros relativos a comederos y bebederos muestran un buen estado de los mismos y diferencias entre explotaciones en los aspectos relativos a la presencia de agua o comida y su limpieza. Existen ciertos indicadores como son los índices de suciedad, de cojeras, estado de la ubre, estado de las pezuñas y de las mucosas, que presentan muy poca variación entre ganaderías e individuos del rebaño en el presente estudio, ya que los animales se encuentran en condiciones de semilibertad en amplios cercados, por lo que los valores de estos parámetros siempre son muy positivos con respecto al BA. Igualmente, los índices de acicalamiento, rumia, decúbito y comer, son muy dependientes del manejo alimentario de cada explotación y no registran variaciones concluyentes entre explotaciones ni entre tipos de animal es muestreados.

Se detectan ciertas diferencias entre las calificaciones medias de los parámetros evaluados en las diferentes explotaciones, como es el caso de los indicadores de relación con el factor humano las prácticas de manejo, propias de cada explotación y el mayor o menor contacto entre animal–humano. Ese es el caso de la distancia de huida, con diferencias entre ganaderías que manejan los animales más o menos. Se comprueba cómo, a pesar de su fuerte carácter y su marcado comportamiento agonístico, evidenciado en una mayor tasa de rozaduras, las señales de miedo ante la presencia del evaluador varían igualmente en función del tipo de animal, detectado diferencias entre los diferentes grupos de animales siendo los cuatreños y cinqueños los animales con más tasa de miedo y distancia de huida frente a vacas y sementales, más acostumbrados a la presencia humana. Los signos de curiosidad se manifiestan en mayor medida en los animales de un año. Finalmente, considerando globalmente los valores obtenidos para los diferentes indicadores, se puede concluir que las 6 ganaderías estudiadas cumplen

los requisitos de BA recomendados, encontrándose los animales en los momentos valorados en unas condiciones óptimas. A pesar de ello, se apreciaron diferencias entre las ganaderías de España y México en cuanto a la condición corporal de los lotes de animales jóvenes, que en el caso de México tienen menos nota, posiblemente por la escasez de pasto durante la estación invernal en las fincas dedicadas al ganado de lidia. Los cuatreños y cinqueños son los individuos que mayor condición corporal tienen en todas las ganaderías, por su manejo de alimentación intensivo, lo que queda igualmente reflejado en unas heces más blandas.

1412 - Documenting and improving welfare in Norwegian cattle

Author: Lars Erik Heggen , Åse Margrethe Sogstad, Silje Johnsgard, Annie Haavemoen, Synnøve Vatn

Objective:
In 2022, a mandatory Animal Welfare Program (AWP) was launched by the entire Norwegian livestock industry. The aim of the program is to map, document, and improve the welfare of cattle.
Material and methods:
The cattle industry in Norway, consisting of two dairy companies and several slaughterhouses, have all agreed on the content and the economic sanctions within the AWP. The AWP included approximately 11 500 dairy, beef and feedlot herds. All herds with more than 10 animals must have an AWP visit at least every 16th month, during which a practicing veterinarian evaluates 16 different animal welfare indicators. The selected indicators are based on Welfare Quality®-assessment protocol for cattle, with four focus areas (feeding, housing, health and behavior), and a few management questions, adapted for Norwegian conditions and the limited time available for the visit. The indicators are scored from 1-3 where score 1 means that conditions are satisfactory, score 2 indicates that improvement is recommended, and score 3 is not acceptable (discrepancy) and measures must be taken to avoid economic sanctions. Additional welfare measures, that are not required by law, is also recorded. The welfare assessment report is documented on AWP's digital platform. Veterinarians must undergo a digital course to conduct AWP visits, a comprehensive and detailed guide assists and calibrates the veterinarians' assessments. As of April 2024, 500 veterinarians are approved to perform AWP visits. The Norwegian Cattle Health Service has developed and operates AWP on behalf of the cattle industry, thus having insight into results and statistics on animal welfare at herd and national level.
Results:
As of April 23, 2024, 11 224 producers are enrolled, and 19 285 AWP visits have been conducted. Approximately 96% of the included herds have had at least one AWP visit, with only small differences according to type of production and geography. On average, 0.11 discrepancy and 0.94 improvement area have been identified per visit so far. Generally, there are only small differences between milk and meat production in the proportion of score 3 and score 2. However, there are some differences when it comes to type of welfare discrepancies and recommended improvements. For both beef and dairy herds, the most frequent welfare discrepancy is water access and poor resting area facilities, particularly for calves and young stock. Dairy herds have a higher proportion of discrepancies related to outdoor exercise, while in beef herds follow-up of sick/injured animals was more frequently recorded. As a result of AWP, 1 679 discrepancies have been addressed and measures taken to improve conditions. The present results also show that more than 90% have at least one additional welfare-promoting measure in their herd. As of April 23, 2024, there are 233 producers who do not have an approved AWP status, resulting in a 40% loss in payment on their milk and meat products. Loss of approved status is the result of lack of AWP visit or that measures have not been carried out to improve unsatisfactory conditions. On a national level, an average of 0.1 discrepancies is identified per visit. However, individual veterinary statistics shows there are substantial differences. For veterinarians who have conducted more than 50 AWP visits, this average varies from 0.0 to 1.19, indicating a significant variation in the approach of veterinarians to AWP.
Conclusions:
The welfare of cattle in Norway is well surveyed and documented through AWP visits. Currently, more than 11 000 producers have had one or more veterinary visits with the

purpose of documenting and improving animal welfare. Generally, the welfare and health of cattle in Norway is very good, and many farmers offer more than what is expected by Norwegian welfare regulations. Larger herds have a slightly higher completion rate, but also a slightly higher likelihood of having discrepancies identified. However, there is reason to believe that there are significant differences between veterinarians and how they approach AWP, indicating a need for better calibration.

1465 - Fecoliths associated sloughed-off tails in New Zealand extensive pasture-based beef cattle: A potential welfare concern

Author: Yolande Baby Kaurivi , Richard Laven, Rebecca Hickson

Objectives

Developing an appropriate and practicable animal welfare assessment protocol requires identifying and selecting suitable welfare measures for each production system. In New Zealand's extensive pasture-based system with high water content, it is not unusual to see cattle with fecal soiling, and some with a ring of hardened feces on the tail or with stumpy/short tails). These hardened fecal rings (fecoliths) result in constriction of blood supply to the distal end of tails leading to tail damage and or sloughed-off tails ("short tails"). The term short tail was coined to differentiate these fecolith-associated stumpy tails from actual broken tails caused by injuries or trauma. It is assumed that tail sloughing off causes pain to the animal like tail docking with a rubber ring or the twisting or tourniquet that requires high force to break a cow's tail. Thus, the research aimed to identify the prevalence of fecoliths associated with short tails on New Zealand beef cows to quantify it as a possible welfare concern.

Material and methods

The "short tail" (fecolths) measure was specifically identified as one of 32 welfare measures for the New Zealand pasture-based beef cattle assessment protocol that was trialed during routine pregnancy scanning of 3366 cows in the race on 25 cow-calf beef farms in the Waikato region (Kaurivi et al., 2020). Descriptive statistics for continuous measures were used to capture central tendency. The Shapiro-Wilk test was used to test for normality, and $\log_{10}(n+1)$ was used to transform those variables that were not normally distributed. The threshold for fecoliths as a potential welfare measure was determined based on z scores.

Results

The results showing an average of 4.2% at the study farms and a maximum of 20.6% of assessed cows at one farm was a concern (Figure 2). A painful condition such as fecoliths that is associated with sloughing of tails was given a poor/unacceptable welfare category where intervention is needed immediately. The thresholds imposed by categorization for poor welfare score (>2%) was 4 times more than the derived threshold of 8.1% indicating a probable much more commonly occurring trait at the beef farms.

Conclusions

The 25 farms in this study do not necessarily represent the prevalence of fecoliths of beef cows throughout New Zealand, however, they were suitable for validating the possible welfare concern that this measure presents. Thus, further research could attempt to determine the welfare impact of fecoliths, the timeframe of a dag formation before tail sloughing off, and how painful it is. Remedial strategic plans that a farmer could implement to mitigate or prevent the occurrence (i.e. washing off the dirt or clearing the hardened fecal balls before the tail sloughs or breaks off) need to be defined. The welfare or management compromise or challenges that are involved in response to this condition need further investigation as well. For instance, the research could classify tail dag or dirty tail scoring on a 3-point scale: 0: no evidence of fecal soiling (clean and dry) to dry with slight fecal soiling, 1: wet with hardening fecal soiling, 2: hardened fecal ball formed around the tail or short tail. The envisioned scale could be used as a tool to caution farmers about the state of tail fecal soiling so that remedial actions can be taken.

1486 - Evaluating effects of heat stress on the efficacy of robotic milking systems

Author: Rajesh Neupane, Sushil Paudyal , Juan Velez, Nancy Charlton, Nelson Rodriguez

Objectives
Heat stress impacts the productivity of dairy cattle in addition to contributing to the overall farm efficiency. The impact is well documented in conventional milking systems, but literature on robotic milking systems is scarce. The objective of this study is to examine the associations between the efficiency metrics of robotic milking systems, milk yield within the robots, and temperature-humidity index (THI) in robotic milking operations.
Material and methods
This retrospective study was conducted using data collected from March to October 2023, at a commercial dairy farm in Dublin, Texas, USA, milking 1500 cows with 22 milking robots in a batch milking system. Temperature and humidity data were collected from the nearest weather station. Robot idle time was defined as the time period a robot is not involved in the milking procedure and was calculated as a sum of intervals between milking two cows. Daily idle time per robot was compared with different levels of daily THI (low <72 THI, moderate 72 to 79 THI, and high >79 THI). Statistical analysis was conducted using ANOVA and associated post hoc tests. The model included days in milk (early, mid, late), lactation number, and THI as independent features and robot idle time, milking speed, milking duration, and milk yield per cow as dependent features.
Results
The average robot idle time during the study period was 220 ∓ 1.27 minutes per day per robot. Robot idle time (minutes; mean∓ SE) was higher in the high THI days compared to low THI days ($P < 0.05$; 192∓ 1.88, 231 ∓ 1.74, 280 ∓ 2.52 for low, medium, and high groups respectively). The idle time per robot was lower in morning milking compared to evening milking ($P < 0.05$; 115∓0.76, 106∓0.75 mins/12hr for morning and evening milkings respectively). Robot idle time was not associated with robot direction, or robot location (front, mid, and back) in the barn. Average milk yield per cow per milking was also reduced with increase in THI ($P<0.05$; 16.31∓0.01, 17.56∓0.01, 20.04∓0.01, for high, moderate, low THI levels respectively). The average milk flow rate (lbs/minute) was also reduced during heat stress ($P<0.05$; 1.98∓0.01, 2.04∓0.01, 2.17∓0.01, for high, moderate, and low THI groups respectively). Furthermore, the average duration of milking (seconds) was also reduced in heat-stressed cows ($P<0.05$; 315.10∓0.25, 323.0 ∓0.17, 334.40∓0.14, for high, moderate, and low levels of THI respectively).
Conclusions
The data from this preliminary exploratory study demonstrate a relationship between elevated temperature-humidity index (THI) values and decreased milking efficiency metrics in robotic milking systems, suggesting that heat stress conditions have a detrimental effect on robot efficiency due to the impact on cow behavior.

1487 - Evaluating the “Welfare Quality®” protocol application in milking cows of the Centre region of Portugal, 2023

Author: Margarida Matos, João Mesquita, Helena Vala, Rita Cruz, Carla Santos, Cristina Mega, Carmen Nobrega, Maria Pereira, Manuel Pinheiro, Sónia Martins, Inês Rebelo, FERNANDO ESTEVES

Objectives
Through the conduct of a survey, the aim of this work was to understand not only the opinion of milk bovine producers associated with Proleite (Cooperative of milk producers) after the application of the "Welfare Quality®" protocol but also to assess the value of the changes made by the implementation of the protocol, the advantages these changes brought to the operation, and the main obstacles to implementing the measures required by the protocol.
Material and methods
The survey was conducted between March and June 2023 with 21 dairy cattle producers associated with Proleite with a total of 1589 milking cows. The questionnaires were individually filled during visits to the farms and completed on-site. Participation was voluntary, and the obtained data were entered and analyzed using Excel software, ensuring complete anonymity. The questionnaire consisted of 16 multiple-choice questions, offering various response options and the opportunity for producers to add other options they deemed appropriate.
Results
The majority of the surveyed farms have between 50 and 70 animals. Most producers (81%) claim to be familiar and understand the concept of animal welfare, observed by the "Welfare Quality®" protocol, finding the available information on this topic sufficient for comprehension. Approximately 62% of the producers agree with the measures required by the "Welfare Quality®" protocol, and 57% state that these measures are easy to implement. When asked about the necessity of implementing measures to improve animal welfare, 81% of respondents agreed to changes in order to comply. General improvement of facilities (48%) and the construction of an outdoor area (38%) were the most commonly mentioned measures.
Regarding whether they had already made changes to comply with the "Welfare Quality®" protocol, 91% claimed they had, with the enhancement of drinking water troughs (81%) and the improvement of calf facilities (57%) being the most implemented measures. A significant portion of producers (33%) did not notice significant improvements in the quality and/or quantity of milk produced. Nevertheless, 62% of respondents admit to having recouped the investment made in improvement actions. Financial requirements were mentioned by 90% of producers as the main obstacle to implementing these measures.
Conclusions
It is concluded that most producers are familiar with and understand the concept of animal welfare and agree with the measures required by the "Welfare Quality®" protocol. Most have already implemented measures; however, although they acknowledge that actions to improve animal welfare can bring benefits to the quality of life for dairy cows, these benefits are not equally perceived by all producers.

Acknowledgments: This work is supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the projects CITAB UIDB/04033/2020, CERNAS UIDB/00681/2020 and GHTM UID/04413/2020.

1488 - Efficiency of mercury, digital, infrared and galinstan-based thermometers in goat and sheep

Author: Ana Carolina Pinheiro, Kelly Grayce Perestrelo , Rodrigo Siuffi Abbud, Jennifer Evangelista de Amorim, Julia Marques Nascimento Freitas, Helen Dias Brandão, Larissa Eleuterio Barros da Silva, Maria Claudia Araripe Sucupira

Objectives

Measurement of body temperature is an essential parameter in the examination of individuals and the herd. The standard reference, the mercury thermometer, was substituted by the digital, due to its prohibition in many countries. Moreover, other equipment has appeared such as galinstan-based thermometer (ecological) and the infrared. However, these options have not been systematically evaluated in ruminants. The overall objective of this study was to evaluate correlation and agreement of obtaining body temperatures in small ruminants (goats and sheep) with noncontact infrared, ecological, and digital thermometer compared with mercury thermometer. Measurement of body temperature is an essential parameter in the examination of individuals and the herd. The standard reference, the mercury thermometer, was substituted by the digital, due to its prohibition in many countries. Moreover, other equipment has appeared such as galinstan-based thermometer (ecological) and the infrared. However, these options have not been systematically evaluated in ruminants. The overall objective of this study was to evaluate correlation and agreement of obtaining body temperatures in small ruminants (goats and sheep) with noncontact infrared, ecological, and digital thermometer compared with mercury thermometer.

Material and methods

A total of 48 ruminants, 24 goats, 24 sheep of mixed breed and various ages had their temperature gauged. The measurements were performed for 5 days, twice a day (7 a.m - 9 a.m. and 1 p.m. - 3 p.m.). The cutaneous temperature were gauged by the noncontact infrared thermometer on the forehead (FH), lacrimal gland (RD = right eye; RE = left eye); armpits (AR = right; AL = left) and perineum (PE). Rectal measurements were performed with digital (DIG), mercury (MER) and ecological (ECO) thermometers. The mercury thermometer was considered the reference standard; hence, the correlation and agreement were evaluated between this method and the others. These values were obtained by the Pearson and Spearman correlation, and Bland-Altman methods. Regarding the correlation coefficient, $r < 0,39$ indicated weak correlation; $0,40 > r > 0,69$ moderate; $0,70 > r > 0,89$ strong and $r > 0,90$ very strong one (SCHOBER; BOER; SCHWARTE, 2018). Relative the agreement, when the standard deviation of the differences (SD) < 0,5ºC and - 0,3ºC > mean of the differences (Me) > 0,3ºC, the T test was performed to evaluate whether the differences were statistically different to 0ºC, the regression analysis between the average and the differences was executed to identify proportion bias ($p < 0,05$). When only SD < 0,5ºC, and the coefficient of determination (R^2) > 0,70, the linear regression equation was calculated.

Results

Body temperatures above 33,8ºC could not be measured by the infrared thermometer, so in these cases data “low” was obtained. This result was recorded mainly in the forehead in the morning, goats (43%) e sheep (41%).Relating the goats, ECO showed a very strong correlation ($\rho = 0,86$) and DIG a moderate ($\rho = 0,66$). In the infrared, PE

obtained the greater correlation and least SD (ρ = 0,49; SD = 0,76). Me from ECO and DIG (Me = -0,03; -0,04 e SD = 0,30; 0,42), were statistically equal to 0ºC, however, both of them demonstrated proportion bias. Only the regression equation of ECO was calculated (MER = 0,479 + 0,9867 ECO) because its coefficient of determination was > 0,70 (ECO = 0,71; DIG = 0,44). Concerning the sheep, the biggest coefficient and smallest SD in the infrared thermometer regions were obtained by PE (ρ = 0,30; SD = 0,79). While ECO (ρ = 0,78) and DIG (ρ = 0,71) showed strong positive correlation. The Me of ECO and DIG (Me = 0,14; 0,09 e SD = 0,31; 0,35) were statistically equal to 0ºC, proportion bias was found in DIG. The regression equation of DIG was not calculated because the coefficient of determination was 0,62 ($R^2 < 0,70$).

Conclusions

Based on these results, noncontact infrared thermometer cannot substitute the mercury thermometer. Although eyes and perineum can be promising regions. The digital thermometer cannot substitute mercury in small ruminants. The ecological thermometer can be a mercury substitute only in sheep. While in goats, it is necessary to use the regression equation.

SCHOBER, Patrick; BOER, Christa; SCHWARTE, Lothar A. Correlation coefficients: appropriate use and interpretation. **Anesthesia & analgesia**, v. 126, n. 5, p. 1763-1768, 2018.

1492 - RECOMBINANT SUBUNIT VACCINE AGAINST BOVINE VIRAL DIARRHEA VIRUS AND BOVINE HERPESVIRUS TYPE 1

Author: Hernan Ramirez Varela, Cristian Dotto, Valentina Carrettoni, Lucia Rocha, Demian Bellido, Jose Angel Martinez Escribano, Viviana Parreño, Andres Wigdorovitz, Pablo Sueldo

Objectives

The aim was to develop a subunit vaccine capable of targeting both bovine viral diarrhea virus genotype 1 subtype a (BVDV 1a) and bovine herpesvirus (BoHV-1), expanding on the Vedevax Block product available in the market since 2018 (Bellido et al., 2021); this vaccine carries the E2 protein of the 1a subtype of BVDV as an antigen linked to a directing molecule called APCH (APCH-E2). With the new formulation, coverage against bovine herpesvirus is added by incorporating another antigen, the BoHV-1 gD glycoprotein (Alves et al., 2014), also linked to APCH (APCH-gD).

Material and methods

The vaccine development followed all guidelines of the National Service for Agrifood Health and Quality (SENASA) and complied with international regulations. The Baculovirus Recombinant system was chosen for expressing both fusion proteins APCH-E2 and APCH-gD. Master and Working banks were established for SF9 cells and the APCH-E2 and APCH-gD baculoviruses, along with respective controls: karyotypes, certificates of freedom from adventitious viruses and mycoplasma, sequence identity, purity, and viability. Antigen production was controlled, as was the vaccine formulation. The vaccine was evaluated in the guinea pig model approved by the health authority for assessing the immunogenicity of bovine viral vaccines (Resolution 598/2012 of the Ministry of Agriculture, Livestock, and Fisheries of the Nation, published in the Official Gazette on December 4, 2012). Five guinea pigs were vaccinated with two doses of vaccine, 21 days apart, subcutaneously. The volume of the dose administered to the guinea pigs was 0.6 ml, 1/5 of the volume of the dose given to bovines. For the dose-response study, serum samples were obtained at 0-, 30-, and 60-days post-vaccination (dpv) to determine the kinetics of the antibody response in the laboratory animal model. Blood extraction was performed via cardiac puncture under anesthesia, following ECVAM recommendations for animal welfare. The protocol was approved by the Ethics Committee of CICV and A, INTA (CICUAE).

Results

Both proteins (APCH-E21a and APCH-gD) were successfully expressed, and their identity was evaluated by western blot. ELISA was used for the quantification of APCH-E21a, while Dot Blot was chosen for quantifying the APCH-gD protein while a qELISA is underdevelopment. The vaccine response in the guinea pig model was highly satisfactory for BVDV 1a, with neutralizing titers greater than 3 (60 dpv), and satisfactory for Bovine Herpesvirus type 1, with neutralizing titers greater than 1.31 (60 dpv). No adverse effects or reactions were observed in the treated guinea pigs post-vaccination.

Conclusions

Based on these results, the new vaccine has been presented and approved by the National Advisory Commission on Agricultural Biotechnology (CONABIA), and currently, experimental batch development is underway alongside the dossier preparation for its

subsequent submission to the National Service for Agrifood Health and Quality (SENASA). Overall, this study represents an expansion in coverage by adding valency against herpesvirus to the existing vaccine against BVDV, offering substantial benefits for animal health and the prosperity of the livestock industry. The integration of advanced biotechnology techniques with comprehensive testing supports the efficacy and safety of the vaccine, paving the way for its potential adoption nationally and internationally. This advancement not only promotes animal health but also contributes to the sustainability and vitality of the livestock industry overall.

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1576 - Pre- and post-transport diet affect the physiology of veal calves undergoing long-distance transport

Author: Susanne Siegmann, Luca L. van Dijk, Niamh L. Field, Gearoid Sayers, Katie Sugrue, Cornelis G. van Reenen, Eddie A. M. Bokkers, Muireann Conneely

Objectives
Every year, large numbers of young calves are transported over long distances from Ireland to Dutch veal farms via ferry and road. Extended fasting periods during transport are a major welfare concern for these calves. Prolonged food withdrawal can result in low blood glucose and negative energy balance and contributes to dehydration and weight loss, which are associated with increased risks of disease and mortality on veal farms. Increasing the amount of milk replacer fed before and after transport may mitigate the adverse effects of fasting on calf physiology during transport and promote quicker recovery post-transport. This study aimed to investigate the effects of pre- and post-transport feeding protocols with different planes of nutrition on the physiological state of un-weaned calves during and for three weeks following long-distance transport.
Material and methods
Two shipments of 58 calves each were transported from an assembly centre (AC) in Ireland via ferry to a lairage (LA) in France and, after a 13-hour rest stop, via road to two veal farms in the Netherlands. At the AC, calves were either fed 2L of milk replacer the morning of transport (pre-CTRL) or 3L of milk replacer the evening before and the morning of transport (pre-ALT). For the recovery phase spanning three weeks after arrival on the veal farm, an additional post-transport treatment was applied, resulting in a 2x2 factorial design. Here, calves were fed according to a standard veal farm protocol (post-CTRL; 1.6L twice daily, gradually increasing to 2.9L) or were provided with 25% greater volume of milk replacer per feeding (post-ALT).
Blood samples and body weight were taken at AC and LA, upon arrival at the veal farm, and 12 and 21 days after arrival. Blood was analysed for variables indicating energy balance (glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB)), dehydration (urea, sodium, potassium, corrected chloride), and (muscular) stress (cortisol, creatine kinase, lactate). Linear mixed models were used to analyse the effects of pre-and post-transport diet, time-point, shipment, and breed on blood variables and body weight.
Results
Pre-ALT calves had lower NEFA (0.10 vs. 0.20mmol/L, $P<0.0001$) and BHB (0.06 vs 0.11mmol/L, $P<0.0001$) levels at AC and LA (NEFA: 0.59 vs. 0.69mmol/L, $P=0.041$; 0.25 vs 0.34mmol/L, BHB: $P<0.0001$), but higher glucose at LA (3.67 vs. 3.32mmol/L, $P<0.01$), and higher NEFA upon arrival (0.83 vs. 0.65mmol/L, $P=0.001$) than pre-CTRL calves. Regarding hydration status, pre-ALT calves had lower urea (2.59 vs. 3.67mmol/L, $P<0.0001$), sodium (143.5 vs. 144.4mmol/L, $P=0.049$), and potassium (6.67 vs. 6.87mmol/L, $P=0.036$) levels than pre-CTRL calves at LA, and corrected chloride was lower for pre-ALT calves overall during transport (95.6 vs. 97.1mmol/L, $P<0.0001$). For stress-related variables, lactate levels were higher for pre-ALT calves at AC (1.58 vs 1.36mmol/L, $P=0.026$) and upon arrival (1.82 vs. 1.54mmol/L, $P=0.017$). Mean values for both pre-transport diet treatments were outside reference ranges for 3/10 blood

variables at AC (and only pre-ALT for lactate), 8/10 at LA (and only pre-CTRL for urea), and 10/10 upon arrival.

During recovery, pre-transport diet continued to influence some variables, with pre-ALT calves showing lower BHB levels on day 12 (0.08 vs. 0.10mmol/L, P<0.01) and 21 (0.09 vs. 0.11mmol/L, P=0.034), and higher lactate levels on day 12 (1.15 vs. 1.01mmol/L, P=0.021) after arrival. Regarding post-transport diet, post-ALT calves had lower mean corrected chloride (96.2 vs. 96.9mmol/L, P<0.0001) and urea (2.61 vs. 2.85mmol/L, P=0.035) over the recovery period, but weighed more than post-CTRL calves on day 21 (61.9 vs. 60.7 kg, P=0.025). Mean values for all treatments were within normal reference ranges for all blood variables by day 12 after transport.

Conclusions

We conclude that offering calves larger volumes of milk replacer before long-distance transport improves their physiological condition during the journey, particularly regarding energy balance and hydration status. However, calves fed more are still impacted by transport and positive effects of feeding are mostly not maintained until arrival. Feeding 25% more milk replacer for three weeks post-transport had limited effects on calf hydration and weight, and normal calf physiology was restored 12 days after transport irrespective of feeding treatment. While pre-transport diet may be a key strategy to enhance calf welfare during transport, further research is necessary to explore means of improving calf physiology throughout long-distance transport that spans several days and approaches to facilitate post-transport recovery.

1617 - Relation of Welfare, Chronic Diseases and Hair Cortisol in Dairy Goats on an Intensive Grazing System

Author: Jesús Iván Ortega Cortés

Objectives

The objective of this study was to relate the presence of chronic diseases, hair cortisol and health indicators according to the AWIN protocol, in dairy goats in agrazing production system in a productive cycle.

Material and methods

An initial group of 70 dairy goats were oestrus-synchronized and only 43 pregnant were selected. Blood simples were taken for diagnosis of leptospirosis by microscopic agglutination; caprine arthritis encephalitis (CAE), paratuberculosis (PTB) and brucellosis by the Luminex technique. In addition, blood, feces, milk and hair (three days before mating, 7 d postpartum, at 60 and 150 d of lactation for blood count, McMaster test, milk quality and cortisol; also, physical examination and welfare assessment using the AWIN protocol for dairy goats, including individual indicators.

Results

Body Condition Score ($P<0.0001$), hematocrit ($P<0.0001$), total solids ($P<0.0001$), fibrinogen ($P=0.0293$), leukocyte count ($P=0.0032$) nematodes ($P=0.0001$), strongilides ($P=0.0070$), coccidia ($P=0.003$), milk density ($P<0.0001$), lactose ($P<0.0001$) and fat ($P<0.0001$), showed differences between some of the production stages. *Leptospira sp.* prevalence of 44.18% was observed, with Icterohaemorrhagiae being the most frequent serovariety (51.7%), followed by Hardjo and Pomona (both 20.7%) and Gripotyphosa with (6.9%), all high pathogenicity serovars. For CAE, positivity in 72%, while for PTB 46.5%. In the cumulative number of chronic diseases in goats, only 6 (14%) were not positive for any of the diseases in this study, 14 (32.5%) were positive for one disease, 22 (51.2%) positive for two diseases, and 1 (2.3%) positive for three diseases. Hair cortisol presented highly significant statistical differences ($P= 0.008$) in different productive stages. While in the comparison between productive stages and number of diseases, the groups with the highest number of diseases presented significant differences regardless of the productive stage. AWIN assessment had satisfactory results in all cases and no differences were observed between the individual health indicators in any period and the number of diseases.

Conclusions

It was identified a relation between cortisol concentration and the number of positive diseases in goats regardless of the productive stage. In the absence of statistical evidence regarding other variables, it is understood that this relationship can be associated with chronic stress typical of the productive stage, however, it is necessary to carry out more studies that rule out other stimuli that raise cortisol concentrations, such as pain.

1625 - Evaluation of the status of biotechnical protection against pests on selected dairy cattle farms in Poland

Author: Ewa Sady, Maria Sady, Karolina Ferenc, Romuald Zabielski, Axel Wehrend, Zdzisław Gajewski

Objectives
Undesirable organisms on the farm can transmit various pathogens and negatively affect animal welfare. Proper bioasecuration is a key element of preventive veterinary measures that positively affect the epizootic status of the herd. With a well-chosen pest-proofing programme for the farm, the risk of spreading infectious diseases can be minimized, animal welfare can be improved and the use of antibiotics can be indirectly reduced, as well as food production route can be positively affected. Biotechnical protection against pests in dairy cattle involves the use of biological methods and technologies to control and manage pests in a sustainable and environmentally friendly manner
Material and methods
The study of assessing the status of biotechnical pest defenses was conducted in 2023 on selected dairy cow farms in Poland, with the aim of reliably assessing the status of biotechnical pest proofing: insects, rodents, wildlife and birds. The first group consisted of farms with 11-50 cows, II: 51-100 cows, and III: 101-200 cows. An audit was performed in ten farms from each group to assess the quality of facility's security. Among other, the condition of fencing, cleanliness in the barns, tightness of the barns, the type of ventilation, implemented protection against rodent infestation, and the use of traps and biocides against flying insects (*Calliphoridae, Muscidae, Ceratopogonidae, Culicidae*) were evaluated.
Results
The study of assessing the status of biotechnical pest defenses was conducted in 2023 on selected dairy cow farms in Poland, with the aim of reliably assessing the status of biotechnical pest proofing: insects, rodents, wildlife and birds. The first group consisted of farms with 11-50 cows, II: 51-100 cows, and III: 101-200 cows. An audit was performed in ten farms from each group to assess the quality of facility's security. Among other, the condition of fencing, cleanliness in the barns, tightness of the barns, the type of ventilation, implemented protection against rodent infestation, and the use of traps and biocides against flying insects (*Calliphoridae, Muscidae, Ceratopogonidae, Culicidae*) were evaluated.
Conclusions
The current state of pest-proofing of dairy cattle farms is mostly influenced by the owners' lack of awareness of the risks posed by pests and lack of knowledge of modern bio-assurance techniques. Farmers are not inspected by quality certification bodies, unlike factories manufacturing dairy products. Implementing a holistic approach that combines various biotechnical measures and adapting strategies based on the specific challenges of the farm can contribute to effective pest management while promoting the health and well-being of dairy cattle. Regular assessment and adjustments to the pest control plan are crucial for long-term success.

1660 - Comparative assessment of peripartum sympathetic activation in primiparous and multiparous dairy cattle

Author: Mark Hiew, Ameer Megahed, Peter Constable

Objectives
To determine whether the sympathetic nervous system is activated in the periparturient period to a greater extent in primiparous than multiparous dairy cattle.
Material and methods
A convenience sample of 106 late gestation non-lactating Holstein-Friesian cattle (34 primiparous, 72 multiparous) were enrolled in the study between May and March. All animals were deemed healthy based on routine physical examination. Animals were moved from an outdoor dry lot to an indoor temperature-controlled individual box stall at approximately 4 days before the estimated parturition date. Cattle were fed typical North American diets for the late dry period and early lactation once daily between 08:00 and 09:30 and had ad libitum access to water. There was minimal opportunity for hierarchical interaction between herd mates during the study. Animals were examined daily at approximately 09:00 from 4 days prepartum to 3 days postpartum. Urine and coccygeal blood samples were obtained, and plasma cortisol concentrations were analyzed using a solid-phase radioimmunoassay. Hematocrit was determined using the microcentrifuge method. Plasma albumin concentration was measured using the bromocresol green method and total protein concentration and urine specific gravity were measured using refractometry. Heart rate (HR) and mean arterial pressure (MAP) were measured from the proximal portion of the tail using a non-invasive oscillometric method and MAP was adjusted for the vertical distance between the site of measurement and the scapulohumeral joint. The time of parturition was recorded to the nearest hour. Data was categorized into 12 h intervals relative to the time of calving for statistical analysis that was conducted using mixed models analysis of variance. $P<0.05$ was considered significant.
Results
Least squares mean plasma cortisol concentration was consistently higher ($P=0.038$) during the 48 h period before parturition in primiparous cattle (10.4±0.8 ng/mL) than multiparous cattle (8.3±0.6 ng/mL). Plasma cortisol concentration immediately after parturition was twice the value 12 h previously. Hematocrit did not differ ($P=0.22$) between groups during the study period but increased gradually from 33.1±0.4 on day -4 to 36.0±0.3 immediately after parturition, and then returned to 33.0±0.3 by day 3. Mean plasma total protein concentration, albumin concentration, and urine specific gravity did not differ ($P=0.34$, $P=0.17$, $P=0.071$, respectively) between groups during the 4 days before parturition. Mean HR during the study period was consistently higher ($P<0.001$) in primiparous cattle (97.7±1.0 bpm) than multiparous cattle (86.2±0.8 bpm) and increased transiently in both groups at parturition. Least squares MAP was consistently higher ($P=0.002$) in primiparous cattle (96.8±1.3 mmHg) than multiparous cattle (91.6±0.9 mmHg) during the 7-day study period.
Conclusions
The hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system are activated in dairy cattle during the 4-day period before parturition. Primiparous dairy cattle appear to be sympathetically activated to a greater extent in late gestation than multiparous dairy cattle, based on their higher plasma cortisol concentrations, HR, and MAP. Our findings suggest that additional studies on stress mitigation in the periparturient period of dairy cattle are indicated.

1679 - CLINICAL EVALUATION OF 3-SITE VERSUS 4-SITE DISTAL PARAVERTEBRAL BLOCKS IN STEERS UNDERGOING STANDING ABDOMINAL LAPAROTOMY

Author: Maya Keefer , Derek Foster, Jennifer Halleran, Margaret Mooring, Kelley Varner

Objectives
Distal paravertebral blocks (3DPV) are traditionally performed at three sites (L1, L2, L4) to desensitize T13, L1, and L2 nerve roots. Many practitioners include a fourth injection site at L3. We aimed to determine if the four-site injection technique (4DPV) improved surgical condition scores and postoperative pain scores in steers undergoing laparotomy.
Material and methods
Steers were randomized into one of two blinded treatments, 3DPV or 4DPV. 2 mg/kg 2% lidocaine was injected at L1, L2, L3, and L4 in the 4DPV group. 3DPV received an equivolume of 0.9% NaCl at L3. Physiologic data, reaction scores, and surgical scores were collected at predefined surgical events. Post-operative pain scores and physiologic data were collected at predetermined time points for 24 hours following surgery. Statistical analysis was performed using a mixed linear regression model to compare intra-operative and post-operative physiologic data between groups. A generalized linear mixed effects model was used to compare surgical conditions and reaction scores. A p-value of <0.05 was considered significant.

Results
The only block failure occurred in the 3DPV group. The only steer who required rescue analgesia was in the 3DPV group. No significant differences between groups were found in intra-operative physiologic data, surgical conditions score, and post-operative pain scores. The 4DPV group had lower respiratory rates following return to stall ($p<0.019$) and one hour following surgery ($p<0.004$).
Conclusions
The addition of a fourth injection site at L3 for distal paravertebrals significantly decreased postoperative respiratory rates in steers following laparotomy. Additional research is required to determine if it improves block success.

1680 - Effect of chronic diseases on animal welfare and hair cortisol concentrations in housed dairy goats

Author: MAYRA SIERRA GARCÍA, IRMA EUGENIA CANDANOSA ARANDA

Objectives

Determine the effect of chronic diseases on animal welfare and hair cortisol concentration in housed dairy goats at different productive stages.

Material and methods

Sixty dairy goats, multiparous, over 4 years, were randomly selected. Health status was assessed using the Luminex® Multiplex test (Najera *et al*., 2023) to detect Small Ruminant Lentivirus (SRLv), *Mycobacterium avium* subsp. paratuberculosis (MAP), and *Brucella melitensis*. Microscopic Agglutination test identified ten *Leptospira spp* serovarieties. Previous results of an outbreak of herpesvirus, caused by *Caprine Alphaherpesvirus* type 1 and *Ovine Gammaherpesvirus* type 2, were provided, diagnosed using the ELISA and PCR techniques (Madrigal *et al.,* 2023). Following etiological identification, goats were grouped by etiologies and monitored throughout the observational period. Blood samples were collected two days before mating, 7 days postpartum, and at 80 and 150 days of lactation for hemogram.

Individual assessments of animal welfare (AWIN, 2015) were conducted at two days before mating, 7 d postpartum, and at 80 and 150 d of lactation. The evaluation focused on indicators related to good health, including body condition, coat condition, abscesses, lameness, nasal and ocular discharge, and fecal dirt. Additional indicators considered for good health included udder alterations, arthritis, mucosal lesions (oral and vaginal), lymphadenomegaly, vaginal discharge, and cough.

Individual milk production was recorded every 15 days. Milk samples were collected manually at 30, 80, and 150 days of lactation. Somatic cell count (cells/µL) and milk composition (percentage of fat, protein, lactose, and non-fat solids) were analyzed.

Hair samples the chest area (10 x 15 cm) was shaved, were collected 2 days before mating, 7 days postpartum, and at 80 and 150 days of lactation. Cortisol concentrations were determined using an adapted cortisol extraction methodology (Koren *et al*., 2002 and Davenport *et al*., 2006 and analyzed by an indirect ELISA test.

Employing SAS® (v 9), our analysis utilized Univariate Proc for central measures, validated data normality and homogeneity with Shapiro Wilk and Bartlett's tests, and applied ANOVA with Tukey's post hoc for inter-stage differences. ANOVA with repeated measurements over time (proc MIXED) was used to evaluate cortisol concentrations and number of diseases per productive stage, the level of significance was $p<0.05$. Chi-square tested qualitative health indicators, while Pearson and Spearman correlations examined associations ($p < 0.05$).

Results

Prevalence were Small Ruminant Lentiviruses (71.66%), *Mycobacterium avium* subspecies *paratuberculosis* (5%), *Leptospira* spp. (40%) and *Herpesvirus* (50%). Goats testing positive for one, two, and three pathogens: 31.67%, 50%, 11.66%. The main haematological alterations that were recorded according to the number of chronic diseases (($p<0.05$) present in the herd, for those with 1 and 2 diseases were hyperproteinaemia (84.94 ± 1.58 g/L) and hyperfibrinogenaemia (6.11 ± 0.65 g/L), also

observed leucocytosis (13.95 ± 0.64 x 109/L) and neutrophilia (8.42 ± 0.44 x 109/L) for goats that had one disease (p<0.05)

Welfare indicators (p<0.05) regarding disease number: poor body condition, coat, udder conformation, mucosal lesions. No significant differences (p>0.05) in hair cortisol concentrations related to chronic diseases in dairy goats. Higher hair cortisol at 150 days of lactation (16.65 ± 1.39 pg/mg) vs. mating (9.55 ± 0.04 pg/mg) (p<0.05), regardless of diseases. No significant association (p>0.05) between milk production, composition, somatic cell count, cortisol concentrations, and diseases.

Conclusions

Seroprevalence of diseases, regardless of clinical signs, did not impact hair cortisol concentrations significantly. However, variations were observed among productive stages, suggesting goats may adaptively manage disease-induced stress without maintaining consistently high cortisol levels.

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1688 - Impact of chronic diseases and hair cortisol on productivity and welfare of stabled dairy goats over two production cycles

Author: MAYRA SIERRA GARCÍA, IRMA EUGENIA CANDANOSA ARANDA

Objectives

Determine the impact of chronic diseases and hair cortisol on the production and welfare of stabled dairy goats over two production cycles.

Material and methods

The study is characterized as an observational, analytical, longitudinal, and prospective investigation (Noordzij et al., 2009). It involved tracking the same animals across two consecutive production cycles (Cycle 1: November 2020 – October 2021, and Cycle 2: October 2021 – August 2022). The study spans the stages of mating, gestation, postpartum, and lactation in goats seropositive to etiological agents of chronic diseases. Sixty dairy goats, multiparous, over 4 years, were randomly selected. Blood samples, obtained two days pre-mating, were processed using advanced techniques like Luminex® Multiplex for the detection of Small Ruminant Lentivirus (SRLv), *Mycobacterium avium* subsp. paratuberculosis (MAP), and *Brucella melitensis*. Serovar identification of *Leptospira spp* was accomplished through the precise Microscopic Agglutination Technique. Previous results of an outbreak of herpesvirus, caused by *Caprine Alphaherpesvirus* type 1 (AHvC-1) and *Ovine Gammaherpesvirus* type 2 (GHvO-2), were provided, diagnosed using the ELISA and PCR techniques. Following etiological identification, goats were grouped by etiologies and monitored throughout the observational period. Blood samples were collected two days before mating, 7 days postpartum, and at 80 and 150 days of lactation for hemogram

Animal welfare assessmen were conducted at critical stages: 2 days pre-mating, 7 days postpartum, and at 80 and 150 days of lactation. Welfare indicators, aligned with AWIN standards, included body condition, coat condition, and the presence of various health issues such as abscesses, lameness, nasal and ocular discharge, and fecal dirt. Additional considerations encompassed udder alterations, arthritis, mucosal lesions, lymphadenomegaly, vaginal discharge, and cough.

The milking process involved a mechanized approach. Recordings of individual milk production were meticulously maintained every 15 days from day 30 to day 150 of lactation. Manual milk sampling at specific intervals allowed for the analysis of somatic cell count, acidity and milk composition (percentage of fat, protein, lactose, and non-fat solids).

In the first production cycle, a preliminary shaving of a defined chest area was conducted a month before the initial cortisol sampling to ensure accurate readings. Hair samples were methodically collected from the designated area at specific time points: 2 days pre-mating, 7 days postpartum, and at 80 and 150 days of lactation. The samples were then processed using an enzyme-linked immunosorbent assay (ELISA) for cortisol concentration determination.

The statistical framework incorporated an array of analyses, including descriptive statistics, repeated-measures ANOVA, Tukey's test for mean comparisons, non-parametric tests like the Chi-square test for individual health indicators, and Kruskal-Wallis test for quantitative variables. A rigorous correlation analysis, specifically

Spearman correlation, was employed to assess associations between various variables. Significance was determined the level of $P<0.05$.

Results

The highest prevalences were observed for SRLv (71.66%), *Leptospira* (40%), and *Ovine Gammaherpesvirus 2* (50%). The most prevalent pathogenic serovars of Leptospira interrogans were Icterohaemorrhagiae at 37.5% (9/24), Pomona at 37.5% (9/24), Harjo at 25% (6/24), and Canicola at 16.66% (4/24).

Goats seropositive for a single etiological agent—SRLv, GHvO-2—and for two etiological agents—Leptospira with GHvO-2—significantly influenced ($P<0.05$) levels of leukocytes, neutrophils, and lymphocytes.

In the second cycle, more cases of ulcerative stomatitis were observed ($\chi2=13.244$, $P=0.039$), with a higher frequency in goats positive for GHvO-2 (50%), SRLv (39.3%), GHvO-2+*Leptospira* (50%), and SRLv+MAP+GHvO-2 (50%). Regarding udder characteristics in the second cycle ($\chi2=18.362$, $P=0.005$), mammary hardening and udder asymmetry were observed in 38.5% of SRLv-positive animals and 18.8% positive for SRLv+Leptospira.

Hair cortisol concentrations were not affected by the presence of diseases and their interactions in stabled dairy goats ($P>0.05$). However, significant differences ($P<0.05$) were observed in cortisol levels according to the productive stage, with higher concentrations at 150 days of lactation (17.10±1.26 pg/mg) in cycle 1 and higher concentrations at mating (17.96±1.31 pg/mg) in cycle 2.

Conclusions

Seroprevalence of various diseases did not significantly influence hair cortisol concentrations, highlighting the adaptive capacity of goats. However, it was emphasized that adaptability does not guarantee optimal productive performance or a state of complete welfare.

1701 - EVALUATION OF ANIMAL WELFARE, SEROPREVALENCE OF DISEASES AND MILK PRODUCTION IN HOUSED DAIRY COWS. PRELIMINARY RESULTS.

Author: Samuel Alejandro Ramírez Anduaga, Irma Eugenia Candanosa Aranda, Vicente Lemus Ramírez, Mayra Sierra García, María Angélica Terrazas Arias

Objectives
OBJECTIVE
The objective of this study was to evaluate health indicators according to the Welfare Quality® protocol, the seroprevalence of leptospirosis; infectious bovine rhinotracheitis (IBR), *Mycobacterium avium* subsp. Paratuberculosis (Map) and bovine viral diarrhea (BVD), in Holstein cows in a stable system.
Material and methods
MATERIAL AND METHODS
68 cows were used, 21 days prepartum. The health indicators of the Welfare Quality® protocol were evaluated. This evaluation includes the presence or absence of alterations such as: eye discharge, nasal discharge, cough, difficulty breathing, alterations at the level of the head, sides, rump and legs, body condition, mastitis, diarrhea, vulvar discharge and lameness (absent, mild and severe). Blood samples were taken for diagnosis of leptospirosis by microscopic agglutination; IBR, Map and BVD by the Luminex technique. The cows were classified as low, medium and high producers according to their daily records. The information was analyzed with multinomial logistic regression and a chi square analysis using the level of production as the dependent variable and as independent variables using the results the evaluation of good health from the Welfare Quality and the positivity to the IBR, DVB, Map and *Leptospira* spp.
Results
RESULTS
Positivity to *Leptospira* spp was observed in 100% of vaccine antibodies (Icterohaemorrhagiae, Hardjo, Pomona, Canicola and Gripotyphosa), and an 89.7%. positivity against non-vaccine serotypes. Regarding the seroprevalence for IBR it was 97.05%; 72.04% for BVD of which 48.52% of the samples were weak positive and 23.52% were strong positive. No seroprevalence for Map. Regarding the welfare evaluations, 85% of the cows presented one or more alterations, of which the most relevant were eye discharge (33.8%), nasal discharge (33.8%), altered body condition (25%) and lameness (23.5%). The average adjusted milk production at 305 days was 10.117 ± 2.335. No association was found with any of the dependent variables (P>0.05).
Conclusions
DISCUSSION AND CONCLUSION
Statistically, no significance was observed regarding the level of milk production, seroprevalence of diseases and health alterations in the evaluation of animal welfare. There are multiple factors that govern the level of production at the time of prepartum, which can also affect the level of production of dairy cows in stables and that were not considered within the approach of the regression equation, such is the case to the evaluation of good nutrition, good housing, good health and good zootechnical management. In this study, it is planned to carry out evaluations at different stages of the production cycle and we can respond to the relationship between BA evaluations, the seroprevalence of diseases and dairy production.
Keywords: animal welfare, diseases, Holstein cows

1717 - Evaluation of Animal Welfare in lidia cattle farms in Spain and Mexico

Author: JUAN MANUEL Lomillos Lomillos PÉREZ, Erika Georgina Hernandez Rojas, Urso Davila, Marta Elena Alonso de la Varga

Objectives
The animal welfare (AB) of cattle under extensive management regime has been very little studied and within cattle, the lidia breed, is very interesting in this aspect due to its ethological particularities and aggressive behavior. An optimized assessment protocol is developed based on the Welfare Quality® project, in order to integrate and evaluate BA in the facilities, in group behavior, in physiological signs and in management.
Material and methods
In this study, 6 different lidia cattle farms have been evaluated, 3 of them located in the Extremadura region (Spain) and 3 in the Querétaro region (Mexico). 10 animals were chosen from each subgroup, formed based on the productive moment and the batches of each livestock farm: mother cows, stallions and batches of animals of different ages. Each BA indicator included in the protocol was rated on a scale from 1 to 5.
Results
The first result of the study was that the data collection methodology developed worked adequately. In general, the 6 farms present an optimal rating in all parameters, although it is worth highlighting differences in terms of the approach distance, being lower in those farms where the animals are handled more frequently, also reflected in curiosity. shown by animals. It is important to highlight that the study reflects the absence of thirst and hunger, both long and short term (body condition and rumen filling) in the 6 livestock farms. Regarding health (breathing and mucous membranes, hoof condition and lameness index), optimal BA values were obtained. There is a certain difference between the livestock farms of Spain and Mexico in terms of the body condition of the animals, being noticeably less in Mexico, where winter is the dry season, with less grass.
Conclusions
The protocol used for the evaluation of BA could be used on any lidia cattle farm, since it is quick, simple and does not alter the behavior of the animals. The parameters related to feeders and drinkers show their good condition.There are certain indicators such as the indices of dirt, lameness, state of the udder, state of the hooves and mucous membranes, which present very little variation between farms and individuals of the herd in the present study, since the animals are in conditions of semi-freedom in large enclosures, so the values of these parameters are always very positive with respect to BA. Likewise, the indices of grooming, rumination, recumbency and eating are very dependent on the nutritional management of each farm and do not register conclusive variations between farms or between types of animals sampled.Certain differences are detected between the average scores of the parameters evaluated in the different farms, such as the indicators of the relationship with the human factor, the management practices, specific to each farm, and the greater or lesser contact between animal and human. It is verified how, despite its strong character and its marked agonistic behavior, evidenced in a higher rate of friction, the signs of fear in the presence of the evaluator also vary depending on the type of animal, with differences detected between the different groups of animals. Adult animalsn (4 and 5 years) being the animals with the highest fear rate and flight distance compared to cows and stallions, more accustomed

to human presence. Signs of curiosity are manifested to a greater extent in the members of flocks of one-year-old animals.Finally, it can be concluded that the 6 farms studied meet the recommended BA requirements. Despite this, differences were seen between the livestock farms of Spain and Mexico in terms of the body condition of the batches of young animals, which in the case of Mexico have a lower grade, possibly due to the scarcity of grass during the winter season in the farms dedicated to lidia cattle. The Cuatreños and Cinqueños are the individuals with the highest body condition in all the livestock farms, due to their intensive feeding management, which is also reflected in softer feces.

1722 - Advanced Technology-Monitored Investigation of the Effects of Heat Stress on Rumination, Drinking, and Locomotory Behaviour, as well as Blood Gas Parameters in Recently Calved Dairy Cows

Author: Justina Krištolaitytė , Ramūnas Antanaitis, Karina Džermeikaitė

Objectives
The objective of the present study was to examine whether heat stress has the potential negative impact on dairy cows, leading to significant declines in their feeding and drinking behaviours, decreased rumination time, changes in their movement patterns, and variations in their blood gas parameters in recently calved dairy cows. The goal of this research was to investigate the effects of heat stress by using innovative technologies such as RumiWatch sensors, and along with blood gas measurements.
Material and methods
The study took place on a Lithuanian dairy farm over the summer period from June 15 to July 8, 2023. The farm held a total of 850 dairy cows. The present study involved the examination of 350 fresh Holstein cows who were in their second or subsequent lactation period and had an average annual milk production of 12,000 kg. The temperature-humidity index (THI) was measured using a SmaXtec climate sensor and a heat stress calculator. The cows were categorized into three groups: high risk of HS—THI > 78 (period: 2023.06.15-06.23), medium risk of HS—THI 72-78 (period: 2023.06.24 – 06.30), and low risk of HS—THI < 72 (period: 2023.07.01-08). The research involved performing RumiWatch sensor (RWS) (Itin and Hoch GmbH, Liestal, Switzerland) tests on cows from June 15 to July 30, 2023, aiming to assess their acclimatization to the RWS during the adaptation period from June 15 to 20, 2023. The registration process of ruminating, eating, drinking and moving behaviour began on June 20 and ended on July 30, 2023, with daily registrations. The parameters of the blood gas were recorded on a weekly basis from June 15 to July 30, 2023.
Results
The results of this study indicate that during the initial week, the risk of HS was elevated (THI > 78), followed by a moderate risk during the second week (THI 72–78), and a low risk during the third week (THI < 72). Significant differences were observed in rumination, with a 17.67 percent increase in the first week (THI > 78) and a 13.80 percent increase in the second week (THI 72-78) compared to the third week ($P<0.001$). The activity level of the cows showed an 11.75 percent increase during the first week (THI > 78) in contrast to the third week (THI < 72) ($P<0.001$), also there was a 12.82 percent increase in activity in the third week (THI < 72) compared to the second week ($P<0.001$). The changes in eating, chewing, and lying times were statistically significant, with regression coefficients and R2 values which range from 0.5323 for eating time changes to 0.8374 for down time. Furthermore, there was found a substantial and statistically significant variations in blood parameters, particularly the average blood urea nitrogen (BUN) levels. Its value increased by 19.75 percent in the week with a THI value more than 78, compared to the week with a THI value between 72 and 78, which had a 16.89 percent enhance ($P<0.01$) in comparison to the urea level. There were no noticeable changes to the other blood parameters.
Conclusions

The data suggests that recently calved multiparous dairy cows are substantially effected by heat stress, which has an impact on the duration of activity, chewing behaviours, and overall activity levels as determined by innovative technologies as well as the blood gas measurements. Additionally, it also affects blood gas parameters in these cows. The research suggests that dairy producers should utilize advanced technology in order to monitor and control heat stress in cows. When the THI is over 78, monitoring changes in activity duration and chewing behaviours is crucial. In addition, farmers should modify their dietary strategies by taking into account the ongoing monitoring of blood urea nitrogen levels.

1724 - Identification of Cryptosporidium spp. in water from dairy stables in the Comarca Lagunera region

Author: Jessica Maria Flores Salas, Reyes Alfredo Carrillo Torres, Francisco Gerardo Veliz Deras, Ramon Alfredo Delgado Gonzalez

Objectives

Detect Cryptosporidium spp. in the water

Material and methods

Water samples from outdoor ponds from 40 dairy farms in the Comarca Lagunera were analyzed. Samples were taken from September to November 2021; each sample of 4 liters was taken from the opposite side of the water inlet to the pond. At the pond's edge, a surface with organic matter was identified; it was slightly loosened, and the water from that area was taken. Previously disinfected plastic containers were used and transferred to the laboratory in a cooler at approximately 4 °C. Whether the pond had a polyethylene rubber cover or was just concrete was noted. If the stable had no water tank, the water collected was from the stainless-steel tank. In the laboratory of the Veterinary Diagnostic Unit of the Antonio Narro Agrarian Autonomous University, Laguna Unit, the water samples were allowed to settle for 24 hours at room temperature. After that time, the supernatant was suctioned with the help of a pump and then filtered with a physical testing sieve opening .0017 in #325 to recover the sediment, then approximately 80 ml. The recovered volume was centrifuged at 2500 xg for 10 minutes in 10 ml tubes with screw caps; the supernatant was removed by suction, and the sediment was suspended in a 5:1 ratio in a 2.5% potassium dichromate solution. The water samples were centrifuged again at 2500 xg for 10 minutes; an aliquot was taken from each 2 ml tube and placed in 2.5 ml microcentrifuge tubes for further study. The solution was allowed to stand for 1 hour, and 50 µL was taken from the bottom of the tube, placed on a slide, air dried, and stained with the modified Ziehl Neelsen (ZNm) technique (Garcia et al., 1983).

Results

Five of 40 water samples were positive (12%) for Cryptosporidium spp. corresponding to 36 pond water samples, no oocysts were identified for the four tank water samples, and the five positive samples came from ponds without polyethylene cover

Conclusions

This study detected cryptosporidium spp in water samples from dairy stables in the Comarca Lagunera region with the modified Ziehl Neelsen technique.

1727 - Objectively monitoring animal welfare across contexts

Author: Sabrina Lomax, Cameron Clark, Peter Thomson

Objectives

When animal welfare is compromised, this is reflected by changes in the underlying structure of their behaviour. A common behavioral diversity metric for animal welfare may provide a method to compare and contrast between and within contexts.

This paper investigates if a common metric can be used to identify when cattle are impacted by these factors, and to assess if this metric can be used to assess severity of different types of stresses and resilience to these stresses.

Material and methods

Cattle behavioural diversity was determined from data generated by accelerometer ear tags from three experiments (1) Separation of calves from cows at weaning for cows and calves; (2) Thermal stress: core body temperature (CBT) of cattle; (3) Pain: dehorning; castration; and de-horning + castration.

Ear tags were attached to each animal, which classified one of six states for every minute (resting; medium activity; high activity; rumination; eating, walking and panting). A classification of 'no stress' vs 'stress' was applied: S: 'No stress' = 7 days pre separation, 'Stress' = 7 days post separation; T: 'No stress' = CBT < 39°C; 'Stress' = CBT ≥ 39°C; P: 'No stress' = 6 days pre procedure, 'Stress' = 5 days post procedure.

For each animal, the proportion of minutes in each hour an animal was classified as undertaking a particular behaviour was calculated. Hourly time budget proportions were analysed to assess the effect of stress input on the specific behaviour.

These proportions were then used to calculate the Shannon-Wiener diversity index (Miller et al., 2020) (H-index) for each animal at each hour of its respective study. H increases with the number of behaviour states exhibited and how evenly distributed they are.

Data was analysed using a linear mixed model with fixed effects for stress level ('No Stress' vs 'Stress'), trait (calf separation; cow separation; thermal stress; and the three surgical stresses); as well as a stress level × trait interaction.

Results

There were highly significant interactions between stress and stressor (all $P < 0.001$) indicating differences in proportion in each of these behaviours between stressed and non-stressed states, but the difference depended on the particular stressor. For both medium and high activity, the proportion of time spent in these states was significantly higher when stressed compared with not stressed, for each of the seven stressor types. For resting, significantly less time was spent in this behaviour when stressed compared with not stressed, for all stressors except calf separation.

There was a highly significant stress level × stressor type interaction ($P < 0.001$) indicating different effects of stress for each stressor. Behavioural diversity (H) was always greater in stress vs 'no stress' situation, and for each stressor type apart from castration, these pairwise differences were statistically significant. The largest effects of stress were seen in the separation stressors, for both cows and their calves.

Conclusions

During periods of stress, animals tended to spend less time resting and more time in medium and high activity, regardless of the source of the stress (stressor). Other traits showed less consistent responses to stress, so may not be as useful as overall indicators of stress.

The periods of stress resulted in a higher behavioural diversity. This is converse to the findings of Miller et al. (2020) where stress resulted in stereotypic behaviours and therefore a loss of diversity in zoo animals. It would appear that the stresses resulted in a regular changing of behaviour, i.e. an animal was not able to 'settle down'. Further investigation of the cause of this is warranted.

1737 - Heat Stress Effects on Dairy Cows Metabolic Profile and In-line Registered Milk Fat-to-Protein Ratio

Author: Akvilė Girdauskaitė, Ramūnas Antanaitis, Karina Džermeikaitė, Justina Krištolaitytė, Ieva Ribelytė, Agnė Bespalovaitė, Kotryna Tolkačiovaitė, Deimantė Bulvičiūtė

Objectives

Recently the accelerated rate of global warming has been detrimentally impacting agricultural zones, with significant effects on the livestock sector. This study's hypothesis posited that heat stress markedly influences the milk fat-to-protein (F/P) ratio in dairy cows, and modifies their metabolic profile. In line with this hypothesis, our study aimed to explore and measure the effects of heat stress on the milk F/P ratio and the metabolic profile of dairy cows, employing in-line registration techniques for precise and immediate data gathering. This research emphasizes the effects of heat stress on dairy cows, specifically when the Temperature-Humidity Index (THI) exceeds 72.

Material and methods

The research was conducted in Lithuania between June 1, 2023, and August 31, 2023. The 1200 dairy cows were kept in a free stall barn and fed a Total Mixed Ration (TMR). Daily feeding times were scheduled at 6:00 and 18:00. Based on THI characteristics, cows were divided into two groups: group 1 had THI values below 72, which indicates the comfort zone; and group 2 had THI values above 72, which indicates a higher risk of thermal stress.

Utilizing the BROLIS HerdLine in-line milk analyzer from Brolis Sensor Technology, Vilnius, Lithuania, the composition of milk, including fat and protein, was determined. Throughout the milking process, the analyzer continually evaluates each cow's milk composition. Blood samples were taken using evacuated tubes without anticoagulants following a general clinical examination (twice per week). To test the activities of GGT and AST as well as the quantities of albumin in blood serum, Hitachi 705 analyzer (Hitachi, Tokyo, Japan) and DiaSys reagents (Diagnostic Systems GmbH, Berlin, Germany) were used. An Rx Daytona automated wet chemistry analyzer (Randox Laboratories Ltd., London, UK) and appropriate reagents were used to measure NEFA levels.

Using capillary blood samples drawn from the ear, the Medi Sense and Free Style Optium H systems (Abbott, Great Britain) were used to measure the plasma levels of BHB and glucose. Every day, the BHB concentrations in the blood samples were measured; all of the samples were taken as a part of a clinical assessment. Samples were taken twice a week, consistently scheduled to correspond with feeding on each farm, and between two and four hours following the delivery of fresh feed to determine the maximum BHB levels.

Results

We saw statistically significant variations in the F/P ratio, albumin concentrations, and glucose levels among the groups. In particular, group 2's F/P ratio increased by 8.6% when compared to group 1 ($p = 0.049$). Furthermore, there was a significant 5.8% drop in albumin levels relative to group 1 ($p < 0.001$) and a 4.2% decrease in glucose levels in group 2 ($p = 0.056$). Results for body temperature, BHB, AST, GGT, and NEFA were not

statistically significant. In particular, body temperature dropped by 2.6% ($p = 0.752$), BHB levels increased by 4.8% ($p = 0.685$), AST level activities dropped by 11.1% ($p = 0.110$), GGT level activities dropped by 20.3% ($p = 0.056$), and NEFA levels increased by 11.5% ($p = 0.337$) in group 2.

Our scientific paper's results section examined relationships between THI and different factors. Humidity and the milk fat-to-protein ratio showed a very weak, non-significant link ($r = 0.043$, $p = 0.447$), and there was also a minor correlation with BHB ($r = -0.046$, $p = 0.417$). On the other hand, there was a marginally negative association ($r = -0.160$, $p = 0.005$) with glucose (GLU), indicating a negative link.

There were no discernible relationships found between body temperature, AST, GGT, and NEFA.

Conclusions

This study uses cutting-edge technology to collect data and emphasizes the effects of heat stress on dairy cows, as measured by a Temperature-Hu-midity Index (THI) above 72. We found that the composition of milk had changed significantly, with the fat-to-protein ratio rising in particular, and that metabolic markers including albumin, glucose, and liver enzymes had changed. Generally, there were poor correlations between metabolic indicators and environmental factors, which may have resulted from the small sample size in the study.

1740 - Band-like Tail Lesions in Danish Holstein and Holstein-mix Dairy Cows

Author: Tobias Volhøj , Cecilie K. Nielsen, Ditte M. Schjermer, Natascha S. Jensen, Benjamin M. Jørgensen, Søren S. Nielsen, Henrik E. Jensen

Objectives
The aim was to determine the prevalence of band-like lesions on tails of Danish Holstein and Holstein-mix dairy cows. The band-like tail lesions were characterized at gross inspection and histopathologically. The study was carried out in selected herds and at a cattle abattoir, all localized in Northern Jutland, Denmark.
Material and methods
A total of 458 tails of dairy cows were examined at the abattoir and in 16 herds a total of 2099 dairy cow tails were included. In the herds, data concerning the cows were registered together with information concerning production, housing, and management. On the collected data, univariable and multivariable logistic regression was used to determine possible associations between the occurrence of band-like tail lesions and characteristic cow and herd variables.
Results
At the abattoir band-like tail lesions showed a prevalence of 23%, and in the herds a prevalence of 25% was present. The lesions, were present as ulcerations or fibrotic bands and were always located on the dorsal surface of the tails, and were situated at an average of 7 cm from the tail tip. The lesions encircled the tails to a varying degree, but in most cases, they occupied half or more of the tails circumference. The data from both the abattoir and the herds showed that the odds of having a band-like lesion was higher with higher parity. Furthermore, having a missing tail tip was associated with a lower probability of having a band-like tail lesion, whereas having mattresses in beds was associated with higher odds for this type of tail lesions, than if sand was used as bedding material. Histopathologically, all the ulcerations were chronic, and contained granulation tissue to a variable degree. In the band-like fibrotic lesions, the epidermis in all cases showed hyperkeratosis with rete-peg formation, and the normal structures of the dermis and subcutis had been replaced by fibrotic tissue showing losses of both hair follicles and glands, i.e. scar-tissue formation.
Conclusions
Band-like lesions are common among Danish Holstein and Holstein-mix dairy cows, occurring with a prevalence of 24%. The lesions are present as ulcerations or fibrotic bands, and are characteristically located approximately 7 cm from the tail tip. The occurrence of tail lesions is associated with higher parities, a missing tail tip (negatively), and the use of mattresses as bedding material. The etiology and pathogenesis of the band-like tail lesions are unknown, and further studies are required in order to determine the impact of the lesions on animal health and welfare.
Funding
The Danish Milk Levy Fund funded this study.

1763 - EFFECT OF OPIOID ANTAGONISTS ON THE MALE EFFECT IN BLACK BELLY SHEEP

Author: Victor Octavio Fuentes Hernandez, Gabriel Ernesto Pallas-Guzman, José de Jesus Hernandez-Rangel

Objectives

The objective of this study was to synchronize flocks of Black Belly ewes and study the male effect with and without opioid antagonist medication

Material and methods

Forty ewes and two Black Belly males from the animal husbandry post of the Universidad Autonoma de Aguas Calientes, Mexico, were used. The two flocks were synchronized with intravaginal sponges containing 75 mg of Medroxy Progesterone Acetate for 12 days. The NX group (n=20) was administered 0.5 mg naltrexone (NX) im every 12 hours starting 48 hours prior to removal of the sponges, and for one day thereafter. The control group (n=20) was administered 0.5 ml saline im every 12 hours starting 48 hours prior to removal of the sponges, and for one day thereafter. The NX group was assigned an aproned male, who received 0.5 mg NX every 12 hr for 12 days to coincide with the duration of intravaginal sponging of the NX group of ewes. The aproned male of the control group received a similar saline treatment. Blood samples were taken from the males every third day for testosterone determination, keeping the males as far apart as possible from each other. 24 hours after removal of the sponges, the designated males were introduced to their group at 8am, 2pm and 6pm. The ewe in oestrus was removed and inseminated with fresh semen obtained with the Fuentes intravaginal device (Fuentes, 2023), using the Fuentes transcervical pipette, the onset of oestrus and its termination were recorded, and at lambing the number of offspring produced by each flock was counted

Results

ewes in Group NX, oestrus began 30 hours after removal of the intravaginal sponges, and 48 hours later all were inseminated. In control ewes their oestrus started 38 hours after removal of the intravaginal sponges and oestrus detection and insemination was completed three days after removal of the sponges. In the NX male, testosterone levels remained stable during the first 6 days, increasing significantly after 8 days of treatment, showing an active libido and detecting oestrus quickly. Testosterone levels in the control male remained constant with no noticeable increase. At lambing the Nx ewes had 2.8 % ewe lambs, while the control ewes had 2.1 % ewe lambs. DISCUSSION Ewes with low seasonality can increase their productivity by manipulating hypothalamic endorphins. It is well documented that administration of opioid antagonists such as naloxone and naltrexone, at different doses, facilitate GnRH and consequently LH and FSH secretion. Small doses of 0.5 mg at 12 hour intervals can increase libido in male goats and rams, and advance the GnRH pulse prior to oestrus, facilitating the presentation and duration of oestrus, promoting fertility and prolificacy

Conclusions

Opioid antagonists may be useful in promoting fertility and prolificacy in productive species such as sheep and goats.

1790 - Practice of calves disbudding in Slovenia

Author: Lena Veren Geč, Rok Marzel , Jože Starič, Jožica Ježek

Objectives

The aim of this abstract is to bring into focus the practice of disbudding in Slovenia. Polled cattle are still a rarity in Slovenia and as such most calves/cows undergo disbudding/dehorning. Disbudding is preferred, as it is done quicker, is less traumatic for the animal and carries less risk for both surgeon and the animal. The Animal Protection Act of 1999 dictates that 'anaesthesia is mandatory for painful procedures in vertebrates', therefore procedures calling for anaesthetics are performed by veterinarians only.

Material and methods

Disbudding is done during the first two months (preferably between 2-6 weeks) of the animal's life, before the beginning of horn growth.
First, the animal is sedated via an i.m. or i.v. application of xylazine. This is done to reduce fear and stress as well as ease of handling, shortening the procedure, and analgesia.
When the calf lays down, local anaesthesia is performed, procaine is applied at the midpoint between the lateral canthus of the eye and the horn base to block the r. cornualis of zygomaticotemporal nerve. Before disbudding, the effect of the local anaesthesia is checked by needle pricks around the horn base.
Disbudding in Slovenia is done with the use of a hot iron while caustic paste use is prohibited by law. The hot iron is pressed against the skin covering the horn buds for approximately 5 seconds, until the skin beneath is copper brown. The aim is to destroy the horn-producing tissue around the horn bud. The wound is covered with a wound care spray.
The animals are also given a NSAID injection before or after the procedure.

Results

Beneficial effects observed in case of combined use of an anaesthetic and analgesic agent are confirmed in multiple studies. Following this procedure minimises stress and pain for the animal and makes it safe and easy for the veterinarian to perform.
To ensure compliance with the law, yearly official veterinary inspections are carried out in all farms in Slovenia.

Conclusions

Our experience with this procedure is positive and we recommend it.

1818 - THE IMPORTANCE OF LYING BEHAVIOUR FOR DAIRY COWS

Author: NORHAN CORTÉS FERNÁNDEZ DE ARCIPRESTE, FRANCISCO GALINDO MALDONADO

Objectives

Analyse individual differences of lying and social behaviour of dairy cows with different availability of cubicles.

Material and methods

This study was carried out at the Center of Agricultural Education from the Higher Studies Faculty, Cuautitlán-UNAM. The protocol was reviewed and approved by the internal Committee for the Care and Use of Experimental Animals CICUAE FESC (C 14-10). Eleven healthy multiparous Holstein dairy cows (631 ± 17 kg SE body weight) of 4 to 6 years of age (5.2 ± 0.1 SE) were housed in an outdoor pen composed of two rows of 10 cubicles, with a concrete floor and a roof above the rows of cubicles. Four video cameras with infrared light were mounted at each corner of the pen at 4 m height to record overnight. The ration was composed of a base of corn silage, alfalfa ad libitum, and oat straw. Concentrate was provided 3× per day, once with the morning ration, and during milking (06:30 and 15:30 h). A repeated measure design was used to study the effect of cubicle availability on lying and agonistic behaviours. Cubicle availability during treatments was controlled by blocking access to lesser used cubicles using a rope tied horizontally at the entrance of the cubicles at the height of 1 m. During the experimental phase, three treatments were established: A 20= 20 cubicles available, A15 = 15 cubicles and A8 = 8 cubicles available. Observations were carried out in three consecutive periods each consisting of 12 days. During each period, treatments were imposed for 4 days each in a random consecutive order to all heifers. Cows were observed daily for 6-h periods. On day one, observations started at 08:00 and finished at 14:00; on the second day cows were observed from 14:00 to 20:00; this process continued consecutively completing a 24-h cycle in 4 days of observation per treatment. The behaviours observed and measured were: Lying (proportion of lying time in cubicles, in alleyways and in total) and frequency of agonistic behaviour (contact aggression). Lying behaviour variable were expressed as proportions of observations, calculated as number of observations per behaviour/total number of scan samplings. Likewise, each behavioural variable was averaged by treatment across the three periods (i.e., the proportion of total lying time per treatment divided by 3 replicates) to compare the effect of cubicle availability. The success index was carried out to have the social status of each cow within the study (success index = number of individuals displaced / number of individuals displaced + number of individuals displaced). Cows with a displacement index between 0.41 and 0.5 were considered medium range, with an index above 0.51 were considered high rank and animals with an index below 0.4 were classified as low rank. Descriptive analyses were performed using the program Minitab for Windows and ANOVA for the difference between treatments.

Results

Total lying and within the cubicles were greater in treatments A20 and A15 than in treatment A8 ($F = 6.61$, $n = 3$, $P < 0.05$), while lying in alleyways was inversely proportional to the decrease in cubicles. that is, it increased as the number of cubicles for the cows decreased. On the other hand, agonistic behaviours (contact aggression)

increased in treatment A8 unlike treatments A20 and A15 ($F = 3.79$, $n = 3$, $P < 0.05$). The results obtained in the displacement index to know the social rank of each cow were the following: in the high rank the cows were found 1, 6, 9, 11, 12 and 15; in the middle range were the cows 2,3,5,7,8,13 and 14 and finally in the low range were the cows 4 and 10.

Conclusions

Most cows try to compensate for cubicles lying time with alleyways as soon as stall availability decreases and act more aggressively to gain entry into them. The hierarchy influences lying times and places; cows of higher social rank lying more in cubicles despite the decrease in availability. However, it was found that some of the medium and low range cows lying in cubicles even with their reduction. So, we can conclude that lying time is essential for cows and they will be lying in alleyways if necessary, and social rank does not have a clear influence on lying areas.

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SOCIO-ECONOMIC ASPECTS OF RUMINANT PRODUCTION

1417 - A survey on culling reasons in dairy cattle farms located in Northern Italy

Author: Valentina Lorenzi, Francesca Fusi, Clara Montagnin, Sara Gabriele, Giorgio Varisco, Luigi Bertocchi

Objectives
Cow culling and mortality are unavoidable and common events in dairy farms. They are influenced by management factors, economic factors and animal health. In addition, they have important implications on animal welfare and on social and economic sustainability. This study aimed at starting to collect the first data on culling rates and most common culling reasons in Italian dairy farms located in Lombardy region, where the 43% of Italian dairy cows are reared.
Material and methods
Seven dairy cattle farms were visited to collect data (referred to the year 2022) on adult cattle culling rate and culling reasons. For this observational study, the data stored within the dairy herd management software were download and read together with each farmer. Only data referred to adult cows (parity ≥ 1) were retained and for each culled animal the following data were registered on an Excel worksheet: i) parity order; ii) days in milk, iii) type of departure from the herd (i.e. sale, slaughter, found dead on farm or on-farm emergency slaughter) and iv) culling or death primary reason (i.e. low milk yield, poor reproduction, somatic cell count/mastitis, hoof health/lameness, metabolic and digestive disorders, injuries, udder defects/conformity, old age, reduction of the herd size and others).
Results
Among the 7 dairy farms, 2 farms had less than 100 lactating cows, 3 farms had around 150 lactating cows and 2 farms had more than 500 lactating cows. During 2022, considering the 7 farms together, 1,898 adult cows were reared. All the animals were Holstein Friesian breed with an average milk yield of 37 kg/day. Considering all the farms, the number of adult cows culled during 2022 was 869, that corresponds to the 45.8% of the adult animals reared. Mean age at culling was 2.3 lactations and on average they were culled within the first 200 days of lactation. The most common culling reasons were: poor reproduction performances (25.8%), other reasons (18.2%), low milk yield (15.7%) and somatic cell count/mastitis (14.6%). The 47.6% of the culled animals exit the herd for being slaughtered.
Conclusions
Based on the descriptive analysis, the interviewed farmers culled their cows mostly for reproduction problems, health issues and production-related reasons. These findings are similar to those reported by others authors in Europe. The present study will be extended to a higher number of dairy herds located in Northern Italy, in order to acquire a broader picture of the Italian situation for better understanding the complexity of the culling reasons and for starting tackling the problem of reduced dairy cow longevity.

1540 - Comparison of pregnancy percentage and total cost of two fixed-time artificial insemination protocols applied in dual-purpose cows in the humid tropics of Mexico

Author: Valentín Efrén Espinosa Ortiz, Juan Antonio Flores Ojeda, Juan Antonio Meza Villalvazo, Mauricio Miguel Estrada

Objectives

Thus, the objective of this paper is to compare the percentage of pregnancy and the total cost per gestation of two IATF protocols in dual-purpose cattle grazing in the humid tropics of Oaxaca, Mexico.

Material and methods

The work was conducted during the months of September 2022 to March 2023, at a ranch in the municipality of San Juan Lalana, Oaxaca, in the Papaloapan region. The climate is Aw2 (h '), warm humid, with average temperature of 25.4 ° C and average annual rainfall of 2,187 mm. The herd is composed of cows F1, in breeding production system. The feeding livestock is based on grazing in 6-hectare paddocks with 6 days of occupation, supplementation with 5 kg of silage of corn per head every third day and mineral salt at a rate of 60 grams per head per day. Were used 76 cows between 50 and 120 postpartum days, with body condition of 2 to 3.5 and having a follicle ≥8 mm or a luteal body. Cows were randomly assigned in two treatments: Treatment 1 (T1; n = 44), IATF using 2 mg estradiol benzoate and 1.9 gr progesterone intravaginal device (DIV) on day 0; on day 8, the DIV was removed and 25 mg of prostaglandin F2-alfa, 1 mg of estradiol cypionate and 400 IU of equine chorionic gonadotropin were applied; 48 hours later, AI was performed and 100 mg of gonadorelin acetate was applied; 30 days later the diagnosis of gestation was made by ultrasonography. Treatment 2 (T2; n = 32) consisted of two IATFs; in the first, the same Treatment 1 protocol was used, but on the 16th post IA, the same DIV (second use) was reintroduced to all cows and was removed on day 23 plus the application of 100 µg of gonadorelin acetate; on the 30th day ultrasonography was performed identifying the empty cows to which 25 mg prostaglandin F2-alfa and 1 mg estradiol cypionate were applied for the second time and at 48 hours the second AI was conducted with application of 100 µg gonadorelin acetate; at 30 days post IA the diagnosis of gestation was made again.

Results

The total cost per pregnant cow was $170.21 and $208.4 american dollars (29 december 2023) for T1 and T2, respectively; being the highest percentage represented by the manpower with 39.33% and 44.37% for the T1 and T2, respectively.

Conclusions

It was concluded that both protocols used have an mid-gestation rate in cows F1 dual purpose in the humid tropics, but the protocol used in the T1 is better and cheaper than that of the T2, being that the reintroduction of the DIV causes a reduction in the percentage of pregnancy and higher cost by manpower.

1806 - SOCIOECONOMIC AND TECHNICAL ASPECTS OF GOAT PRODUCTION IN GUANAJUATO, MEXICO

Author: América Alejandra Luna Estrada, María Denisse Montoya Flores, Tomás Arturo González Orozco, Yumi Elena Ghinis Hozumi

Objectives

The study's goal was to show some characteristics of the goat farming activity in the state of Guanajuato, Mexico, by small producers, who received technical assistance (TA) and training during 2013-2022, and the physical, technical, social, and economical resources, they had access to.

Material and methods

The information of 701 static diagnosis questionnaires, from a total of 1222, was evaluated. These were applied between 2013 and 2022 to goat farmers who received support –through assistance programs for small producers– from the Government of the State of Guanajuato (in conjunction with the Federal Government until 2018). During this period, the INIFAP carried out evaluation, follow-up, and training activities of producers and agents of change with the support of the Groups for Livestock Technological Validation and Transfer (GGAVATT, in its Spanish acronym) model. The information was registered in an Excel database (Office Microsoft®) and analyzed to obtain descriptive frequencies and percentages.

Results

The goat farming activity was distributed in 22 municipalities: Acámbaro, Abasolo, Apaseo el Grande, Apaseo el Alto, Celaya, Comonfort, Cortazar, Cuerámaro, Huanímaro, Irapuato, Jaral del Progreso, Juventino Rosas, León, Pénjamo, Salamanca, Salvatierra, San José Iturbide, Santiago Maravatío, Tarimoro, Villagrán, Valle de Santiago and Yuriria, which correspond to the 47.8% of the state.

The producers were aged 19 to 85 years old, with 31% women and 69% men. The time they had received TA and training was from 0 to 12 years and 79.6% of them belonged to a local livestock association. Academically speaking, 85.9% could read and write, while 9.7% couldn't; 20.1% concluded elementary school, 1.9% middle school, 1% high school, 0.1% had a technical career, 1% had a degree, 7.3% had no school education at all, and the rest didn't give this information. For 43.1% of producers, goat farming was the sole source of income; 12.8% employed family members permanently and 3.1% eventually, but only 11% of them were given an economic remuneration. Production and economic records were kept by only 13.7% and 5.4%, respectively. The main product generated was milk (82.7%) under the intensive (39.1%), semi-intensive (57.1%), and extensive (1.4%) systems. The herds were mainly composed by the Saanen (29%), Creole (9.7%), Alpine (8.6%), Toggenburg (0.7%), and Boer (1.4%) breeds, and 46.1% of the producers did not specify. In infrastructure, 25.3% had handling pens and pens to organize their animals by productive stage; 8.3% had a milking parlour or milking area; 56.1% had water troughs and 61.2% had food troughs in the pens; 2.6% had meadows or pastures; 19% had a storage room; 2% had a dairy products atelier, and 2.1% had silos. As for equipment and machinery, only 10.4% had a plough (24.1%), a tractor (19.1%), a truck (34%), a trailer (10.4%), a silage machine (2.9%), a forage baler (2.9%), a milking machine (7.3%), and nipple buckets (1.6%), among others. The marketing of the milk or its derivatives was through sale to collection centers (13.6%), directly to the end consumer

(12.8%), to grocery stores (5%), to the transformation industry (2%), to intermediaries (22.5%), to other producers (0.3%), and other not specified means (2.3%).

Conclusions

Most of the goat farmers in 22 municipalities of Guanajuato, under this organization scheme, worked with the semi-intensive system with limited technical and material resources. The producers had basic level of education. The most producers used family workforce. In the herds, the milk producing animals predominated, being milk the main product generated. Producers carried out the activity mostly on a low scale. These results are similar to those reported by Aréchiga *et al*. (2008) and Lavalle *et al*. (2022), who characterized the goat production systems in Mexico. However, the implemented assistance programs for small producers have had an important role in the development of the activity since Guanajuato currently contributes with high production of goat milk in the country.

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1429 - BIOPROCESSING OF ORGANIC WASTE FOR THE LARVAL DEVELOPMENT OF Hermetia illucens AND SUSTAINABLE PRODUCTION OF PROTEIN SUBSTRATE FOR FEEDING RUMINANTS

Author: Juan Reátegui, Grazia Valeria Reátegui-Rios, Alexander Obando, Ximena Barriga

Objectives

Given the globally rising prices and limited supplies of fishmeal, fish oil, soybeans and other protein sources, there is an urgent need for the feed industry to investigate the possibility of using alternative sources of protein and fat in the diet of productive animals. Insects are a good source of proteins, lipids, vitamins and minerals; they show a small ecological footprint and a high feed conversion efficiency, therefore the use of insect meal to replace traditional protein sources is becoming more and more common in the food industry for animal production. The objective of this research was to produce a protein substrate based on *Hermetia illucens* larvae fed with various organic waste and to study the physicochemical analysis of larval flour from the same, for its use as a protein substrate in the search for an alternative protein source for the production of feed for the feeding of ruminants.

Material and methods

Hermetia illucens is a saprophytic generalist detritivore species that colonizes, grows and reproduces in a wide variety of plant matter and organic waste. Its breeding potential is related to the biological characteristics of its larval stage, able to reduce from 60% to 90% of the volume of organic matter in substrates, it has a bioconversion rate of 140%, with larvae that consume their own weight in food every 12 h, accumulating around 40% or more as protein. Five repetitions of *Hermetia illucens* larvae flour were obtained of the fifth larval stage and prepupa, and were physicochemically characterized for the analysis of its content of Dry Matter, Moisture, Crude Protein, Ethereal Extract, Ash and Crude Fiber according to the AOAC International method. Moisture was measured by drying the samples at a constant temperature of 105 °C for 6 hours or until a constant weight was reached. Crude protein (N×6.25) was analyzed by the Kjeldahl method after acid digestion with an autodigester (FOSS, Tecator, Hoganos, Sweden). Crude lipid was determined via the ether extraction method using the Soxtec System HT system (Soxtec System HT6, Tecator, Sweden). The ash content was determined in a muffle furnace operated at 550°C for 6h. Crude Fiber was determined according to ANKOM.

Results

The physicochemical characterization of *Hermetia illucens* larvae flour reports: Humidity 3.61±0.12%; Dry Matter (DM) 96.39±0.12 %; Crude Protein 54.52±0.86 %MS; Ethereal Extract, 16.82±0.35 %MS; Ash 12.55±0.32 %MS; Crude Fiber 8.48±0.50 %MS; Nitrogen-free extract 7.62±1.11 %MS and Energy 534.95±0.12 Kcal/g. The protein content in *Hermetia illucens* larva meal is higher than Soybeans, 41.10%, Beetles, 42.20%, *Eristalis tenax*, 40.90%, *Tenebrio molitor*, 38.30% and Crickets, 32.60%. The ethereal extract is 16.82±0.35 %DM, there is evidence that this parameter presents variations depending on the food on which the *Hermetia illucens* culture is developed, different authors report a range between 16.10 to 35.00 %DM.

Conclusions

We conclude that *Hermetia illucens* larva meal should be considered as a substrate with a good contribution of protein and Ethereal Extract, a source of protein of animal origin, and as an alternative for the use, in replacement of other traditional sources in the feeding of ruminant and productive animals, with an important foundation for the generation of a circular Bioeconomy.

1438 - Convergence of Sustainable Development and Livestock Research: A Bibliometric Analysis

Author: Fernanda Yazmin Aguilar Cruz, Georgina Hernández Rojas , Juan Nava Navarrete

Objectives

Sustainable development has firmly established its position on the global political, economic, social, and research agendas. Its significance lies in the pressing need to transform socio-economic systems, with the premise of meeting current needs without compromising future resources. In this context, livestock presents a complex issue when it comes to sustainability. On one hand, livestock plays a fundamental role in food security by providing protein-rich foods such as meat and milk. Additionally, it significantly contributes to the economy, generating income and employment, especially in rural areas. According to Herrero et al. (2009), in developing countries, extensive, intensive, and mixed livestock production systems contribute approximately 50% to global meat production and 41% to global milk production. However, in recent years, livestock systems have faced increasing production pressure driven by a notable rise in demand for animal products. This production intensification has been linked to negative environmental effects such as deforestation, greenhouse gas emissions, and the intensive use of natural resources like water and land. In the academic and research sphere, the analysis of sustainable development and livestock has historically evolved independently. Nevertheless, in recent years, growing evidence suggests the convergence of these two concepts, primarily in addressing environmental issues with lesser emphasis on social and economic dimensions. This convergence marks a significant shift in research perspective, highlighting the interrelation of livestock and sustainable development in new research agendas.

Material and methods

The research question guiding this study is: What kind of thematic convergence is observed in research agendas on sustainable development and livestock? To address this question, we applied a systematic bibliometric analysis methodology. Our main objective is to analyze the links, synergies, and mergers between research agendas on sustainable development and livestock. We conducted this analysis by identifying and comparing the structures of the most representative keywords and their co-occurrence in scientific documents. The selection of the document corpus were carried out using the PRISMA methodology (Page et al., 2021). The bibliometric database used was the Web of Science (WoS). The search criteria included the terms "sustainable development" and "livestock," "beef," "cattle," "sheep*," "goat*," and "small ruminants." Through automated indexing tools, we filtered the period, document type, language, and indexing categories. This process led to the final identification of 7,837 documents.

Results

Results regarding thematic convergence include identifying countries with the highest scientific production, the most influential authors, as well as forming networks of words, thematic clusters of co-occurring keywords, and the evolution map over time. In the analyzed period from 1987 to 2022, there is a growth in the annual publication rate exceeding 22%. Of the 7,837 reviewed documents, 81.65% are scientific articles.

Journals with the highest number of publications in the domain were "Sustainability", "Animal", "Agricultural Systems", "Journal of Cleaner Production" and "Journal of Animal Science". The countries contributing the most to the research field are the United States, the United Kingdom, China, Brazil, and Australia. In the co-occurrence network analysis, three thematic clusters are identified. The first is oriented towards the management of livestock enterprises and their relationship with sustainability dimensions. The second focuses on the complex dynamics resulting from the relationship between livestock and the natural system. The third concentrates on the productive performance of livestock species, addressing the production of meat and milk. In the thematic evolution map, four time sub-periods are analyzed. In particular, the period between 2019 and 2021 shows a thematic convergence focused solely on two concepts: "Cattle" and "Management." This is attributed to a widespread decrease in activity due to the COVID-19 pandemic. Subsequently, in the following periods, a greater diversity of themes is observed, leading to the consolidation of new topics and concepts of interest. Aspects such as "in vitro", "conservation", and topics related to "growth" stand out.

Conclusions

In conclusion, the analysis results reveal thematic convergence through synergistic collaboration among interdisciplinary researchers. Undoubtedly, the concept of sustainable livestock stands out as the most significant fusion in this field, emerging from the convergence of various research agendas. However, the literature suggests that in the coming years, areas such as climate change, biome conservation, and biodiversity are projected to further drive research in this field.

1439 - The effect of heat stress on adaptive immunity, metabolic, inflammatory reactions and oxidative stress in dairy cows during the dry period.

Author: Mehmet Akköse, Halit Kanca, Tuğrul Hoşbul, Sema Ören, Mert Pekcan, Murat Onur Yazlık, Esra Canatan Çolakoğlu, Hasan Karakuş, Mehmet Rıfat Vural

Objectives
The aim was to investigate the effects of exposure to heat stress during the last two months of gestation on metabolic and oxidative stress parameters, cytokine release, and helper and regulatory T lymphocyte levels in cows, and to determine any correlation between alterations in these parameters of the dam and the T lymphocyte and serum IgG levels of the calf during the first 24 h after birth.
Material and methods
Thirty-two Holstein cows were randomly assigned to two groups, one of which was exposed to heat stress and the other of which was housed at normal seasonal temperatures in a building with a mechanical cooling system during the last two months of gestation. Blood samples were taken from each cow at 18-21 days before the expected date of parturition and at the time of parturition, and from their newborn calves before and 24 h after colostrum intake. The cows were also sampled for colostrum. Blood samples were tested for Th1, Th2, Treg and gamma/delta T lymphocyte (γδ T) levels using a flow cytometer, and for IFN-γ, TNF-a, IL-17A, IL-10, IL-6, IL-1 beta, nitric oxide, paraoxanase, total oxidant capacity, total antioxidant capacity, SOD, GSH-Px, catalase, MDA, retinol and tocopherol by the ELISA method. ELISA testing was also used to measure calf serum IgG and colostral IgG and lactoferrin levels. Serum samples were analyzed for albumin, total protein, cholesterol, bilirubin, and creatinine levels with the aid of an autoanalyzer. The statistical analysis of the study findings was performed using the linear mixed model, simple effects tests (Bonferroni-corrected), Student's t-test, Pearson's correlation coefficient, Mann-Whitney U test and analysis of variance (ANOVA).
Results
When compared to the group provided with mechanical cooling, the group of cows exposed to heat stress presented with a shorter gestation period (on average 6 days), lower retinoic acid and alpha-tocopherol levels ($p < 0.05$), higher IL-6, IFN-γ and IL-10 levels ($p < 0.01$), higher total oxidant capacity and SOD activity ($p < 0.05$), significantly higher Th1 levels ($p < 0.01$), lower γδ T lymphocyte and Treg levels ($p < 0.01$), and lower colostral IgG levels ($p < 0.05$). Calves born to the cows in the groups exposed to heat stress and mechanical cooling were observed not to show any statistically significant difference for T lymphocyte and serum IgG levels.
Conclusions
It was determined that heat stress induced not only inflammatory reactions, but also anti-inflammatory reactions. Further genetic research is needed to determine the effects of heat stress on calves after the first 24 h of life, and to investigate their thermotolerance levels

1517 - Hemp cultivation for Sustainable livestock farming

Author: Alfonso Gallo , Augusto Siciliano, Luigi Jacopo D'Auria, Giuseppe Rofrano, Gabriele Di Vuolo, Maria Giovanna Buonomo, Donato Sansone, Alessandra Esposito, Antonio Di Francia, Francesco Serrapica, Felicia Masucci, Domenico Vecchio, Giovanna Cappelli

Objectives

Hemp (Cannabis Sativa L.) is a plant that in recent years has seen considerable interest in the industrial, pharmaceutical and food sectors. As for the food sector, a variety of products can be obtained from the plant, in particularly seeds and oil are of growing interest. As for the livestock sector, the Commission Regulation (EU) 2017/1017 of June 15, 2017, amending Regulation (EU) No. 68/2013 on the Catalogue of feed materials, allows the use of the hemp seeds, oil and protein cakes (obtained from the seed after oil extraction), fiber and flour (obtained by milling the leaves), while the inflorescences cannot be used either as feed material or for human food. Hemp varieties that can be used for feed production must have a delta9tetrahydrocannabinol (THC) content of <0.2%. EU legislation sets THC limits in the food sector in ppm, which is significantly lower than the limit imposed on the raw material. Cannabinoids are produced in the glandular trichomes found on inflorescences and to a lesser extent on leaves. In seeds, cannabinoids are present as processing contamination due to contact with the glandular trichomes of inflorescences and leaves. It is therefore inferred that the risk of presence of THC, as well as other secondary cannabinoids such as cannabidiol (CBD), is significantly greater in leaves than in seeds.

It is pointed out that of the use of hemp seeds as livestock feeds is in competition with human use, whereas the leaves, which are not allowed as human food, can also be harvested from cultivation for other purposes and used as forage, thus making use of a production waste.

The study aims to evaluate the quality of hemp leaves from industrial cultivation as a raw material for animal feeding.

Material and methods

The hemp was produced in a farm in Southern Italy from a crop for industrial purposes (no food); the germinated seedlings were planted in early May 2023 and harvested mechanically after 4 months. The plants were dried in cells at a temperature of 17°C and 30% RH in the dark for 20 days. Then the leaves were separate from the inflorescences and branches using special machines.

Results

The resulting product consisted only of dried and ground leaves on which analyses were carried out to determine cannabinoids, aflatoxins, at the chemical laboratories of IZSM (Istituto Zooprofilattico Sperimentale del Mezzogiorno) and chemical composition at the Department of Agriculture, Science University of Naples Federico II. Samples showed a THC content of 0.005g% and CBD content of 1.51g%, and no detectability of aflatoxins B1, B2, G1 and G2. Regarding the chemical characteristics, the product showed a dry matter content of 87.95%, protein 23.67%, fat 11.86%, ash 4.31%, and Neutral Detergent Fiber 20.18%.

Conclusions

These preliminary results indicate that hemp leaves derived from cultivation for industrial purposes (fiber, biomass) can be used as a raw material for ruminant feeding, thus representing an alternative to traditional feeds and promoting sustainable agriculture, as hemp cultivation requires limited amount of irrigation water, no pesticides, and less mechanical labor, it improves the chemical-physical characteristics of the soil, has a natural herbicidal action, and it's a plant that absorbs significant amount of CO_2.

1538 - Estimated greenhouse gas emissions from grazing cows for producing 1 kilogram of milk

Author: Edith Miranda Martínez, Iván Carrisoza Urbina, Andrea Wingartz Otaduy

Objectives

The objective of the present work was to estimate CO_2 eq (Carbon dioxide equivalent) emissions generated by the production of 1 kilogram of milk in a production unit in the Mexican highlands using the formulation provided by the IPCC (Intergovernmental Panel on Climate Change) to determine the primary sources of emissions.

Material and methods

The present work was realized in a herd of dairy cattle in the state of Queretaro, Mx. To characterize the production unit, four years of zootechnical records were considered.

- Characteristics: Holstein, Jersey and crossbred cows were classified into the following productive stages: lactation, growth, development cows in production and dry cows. These are differentiated by age, weight, diet, and reproductive status. The average number of days of milk production in the herd was 358, with an average daily production per cow of 11.8 kilograms. Milk production analyzed and standardized for protein and fat content at 3.3% and 4.3%, respectively, was 302 kilograms of milk per herd. In addition to milk production, parameters considered important were obtained, such as: fertility (30.62%), days dry (68), calving interval (14 months) and days open (152).

- Feeding: the amount of dry matter provided in the diets of each stage was considered, as well as their energy, nitrogen, ash, and protein content.

- Manure management: four types of excreta management in the herd were classified: solid storage, liquid storage, pasture distribution and/or dry lot. However, each stage mentioned above will have one or more of these.

- Greenhouse gas (GHG) estimation: for this project, chapters 10 and 11 of guideline No. 4 reported by the IPCC, entitled “Emissions from livestock and Manure Management” and “Nitrous Oxide Emissions from Managed Soils” were consulted. In accordance with the guidelines, CO_2 equivalents for methane and nitrous oxide of 27 and 273, respectively, were considered.

Results

The total GHG emissions from all stages per year were 554,287.26 kg CO_2 eq, of which 77% came from methane generated by enteric fermentation, this being the main emission category, while the remaining 23% was generated by excreta management (considering methane and nitrous oxide).

Specifically, when examining the result of enteric fermentation emissions, we determined that the cow producer stage has the most significant impact with 328,202.68 kg CO_2 eq of enteric fermentation. This means that each cow has an emission factor of 127 kg CH_4 (methane) per head per year. Under similar conditions, the IPCC assigns 93 kg CH_4 per head per year; this difference is a consequence of the difference in the digestibility of the diets. In the case of the IPCC, digestibility was 77% (IPCC,2019), while in this work it was 62%; this decrease in digestibility causes an increase in methane emissions. This is consistent with the findings of Bente Aby et.al (2019), who evaluated

the effect of feeding grass silage at different stages of maturity and found that ruminant methane emissions were lower when the forage had higher digestibility.

On the other hand, emissions from excreta management were 126,280.04 kg CO_2 eq. 52% were emitted as methane and 48% as nitrous oxide. As with enteric fermentation, cows in production were the major contributors.

Finally, when allocating total emissions (554,287.26 kg CO_2 eq) to total milk production (302,274.92 kg of milk), we obtained 1.83 kg CO_2 eq produced per kilogram of milk. This result is within the range (1 to 2 kg CO_2 eq) according to the study conducted by author Durk Nijdam et. Al. (2012), where he analyzed the emissions generated by one kilogram of the finished product in 12 production systems distributed in Europe and North America.

Conclusions

As Aby and coworkers did (2019), it is possible to assess the effect of increasing dry matter digestibility on total GHG emissions in this herd would be pertinent.

Another alternative that could be analyzed is the increase in the fertility rate, by reducing the days to open to 85-100 and production days to 305, (Gasque, 2008). With this, the kilograms of milk produced could be increased and emissions could be distributed among more product. This strategy is focused on reducing emissions intensity (kg CO_2 eq/kg of milk) but not total emissions. In this way, production efficiency is sought by emitting less (GHG) per kg of milk.

1564 - Interaction between animal welfare and environmental impact: a pilot study in dairy cattle farms

Author: Valentina Lorenzi, Mariana Roccaro, Angelo Peli, Giorgia Liberati, Federica Mattacchione, Alessandra Bonoli, Sara Gabriele, Simone Gallese, Elena Ciaffaroni, Francesca Fusi, Giorgio Varisco, Luigi Bertocchi

Objectives

Life Cycle Assessment (LCA) is widely used to study, measure and quantify the environmental impact of livestock production for better understanding the nature and the severity of the consequences and finding the right mitigation strategies. The United Nation's Intergovernmental Panel on Climate Change (IPCC) included animal welfare among the areas that can be both negatively and positively affected by the application of environmental mitigation measures. Thus, in order to achieve an overall improvement of the sustainability of livestock production, it is important to understand the synergies and antitheses between animal welfare and environmental sustainability. This pilot study is a first attempt to investigate the interaction between animal welfare and environmental impact in dairy cattle farms belonging to the Grana Padano cheese supply chain.

Material and methods

During 2023, 4 dairy cattle farms suppling milk for the Grana Padano cheese production were visited to collect all the necessary data for the implementation of a “cradle-to-farm gate” LCA. In fact, milk production at farm level is estimated to have a very important environmental impact within the cheese production chain.

The farms were located in the North of Italy, in the area of the Grana Padano PDO (protected designation of origin). Data were referred to the years 2021 and 2022. Animal welfare data were obtained from the ClassyFarm system of the Italian Ministry of Health (www.classyfarm.it), while farm milk quality data were provided by the cheese factory.

LCA was carried out using SimaPro software (PRé Sustainability, Netherlands). The LCA model was developed according to the International Organization for Standardization. The functional unit chosen was 1 kg of fat- and protein-corrected milk. In addition, 1 ha of occupied area was selected as a second functional unit, in order to estimate the environmental impact intensity on the land occupied by the farms. An attributional approach, which considered from cradle to farm gate system boundaries, was adopted. Inputs (e.g., fuel, electricity, water, organic and mineral fertilizers, pesticides, off-farm feeds and bedding, drugs, detergents, disinfectants, etc.) and outputs (emissions to air, water and soil and milk), involved in the milk productive process, were considered. Primary data were collected by direct interviews of the farmers, while secondary data were obtained from Ecoinvent version 9.2 and grey literature. The impact was calculated for the yearly production of the farm and the functional units and it was expressed in terms of kg CO2eq for each process included in the model.

Results

The 4 dairy farms had a median of 253.5 lactating cows (63-285). Together they supplied about 11,400,000 kg of milk per year. All the animals were Holstein Friesian breed. Three out of the 4 farms had a biogas plant. All the farms had a photovoltaic system. For each farm the following input data were collected, organized and analyzed: farm size, crop management, herd management, animal health and welfare, drug usage, milk

production and quality, livestock waste management, fertilizer and pesticide use, agricultural equipment and machines, electricity and energy use, water use.

In order to properly assess the various components of sustainability and their interactions, good quality scientific information on each of them was needed, thus data collection proved time-consuming and not all the farms could provide all the information requested, in particular for water use, which was estimated using specific scientific literature. Data analysis is currently underway.

Conclusions

The study of the interaction between animal welfare an environmental sustainability is an urgent, difficult and necessary challenge. Particular attention must be paid to the proper collection of the data, their organization and analysis in order to have comparable scores for the different areas of (i) animal welfare, (ii) antimicrobial use, (iii) environmental impact and (iv) water footprint, so that the positive and negative interactions between these aspects of sustainability can be better understood. Despite consumers' demands, it is undoubtedly very difficult for the current livestock farms to implement independently a robust environmental impact monitoring.

This study was supported by the Italian Ministry of Health grant PRC IZSLER 2022/003

1662 - Effect of brown seaweed supplementation on milk production and ammonia emissions from urine and dung in grazing dairy cows.

Author: Ignacio Beltra´n , Francisco Salazar, Marta Alfaro, Joaquin Beltran, Alejandro Buschmann, Emilia Ferrada, Sara Hube

Objectives

The aim of this study was to evaluate the effect of brown seaweed (*Macrocystis pyrifera*) supplementation on milk production and ammonia emissions from urine and dung in grazing dairy cows

Material and methods

The study was carried out during Spring 2022 (November to December), at Instituto de Investigaciones Agropecuarias, INIA Remehue (40°31´S; 73°03´W; 65 m above sea level) in Osorno, Chile, for a total period of 63 days, where the first 21 days were considered as adaptation period. Thirty-six Holstein Friesian lactating dairy cows were grouped (n=12) according to milk production body weight, days in milk and body condition score. Each group was randomly assigned to one of three experimental treatments: 1) Control: Diet composed by 75% of pasture + 25% of grain-based concentrate, 2) Low seaweed (LSW): Control + 1.5 % inclusion of *M. pyrifera* (Low seaweed, LSW; 125 g dry seaweed/ cows/d), and 3) High seaweed (HSW): Control + 3 % inclusion of *M. pyrifera* (High seaweed, HSW; 250 g seaweed/cow/d). Algae were included in replacement of concentrate and all treatments were iso-energetic and iso-nitrogenous. Seaweed was supplemented on concentrate during morning and afternoon milking. Milk production was daily measured using an electronic meter. Milk samples were taken on week 5, 7 and 9 of experiment, for chemical analysis of protein, fat, lactose and urea. Urine and dung (bulked sample per treatment for each excreta) were collected in the same week of milk sampling, and immediately frozen. Ammonia emissions from urine and dung were measured using dynamic chamber, in a randomized block design (n=4), under controlled conditions (80% water field pore space, 20 °C) for 30 days. All treatment were applied to equivalent rate to 50 kg N/ha. The air was scrubbed clean of ammonia by bubbling through 100 mL of 0.02 M orthophosphoric acid, and then was passed through the chamber containing the treated soil before passing through a similar acid trap to capture ammonia. The acid traps were changed at 2, 4, 8, 24 h, then every 24 hours for the first week, and every 2 days until day 21. Ammonia was determined using automated colorimetry.

Effect of treatments on milk production and ammonia emissions (urine or dung per treatment) were determined using mixed models in SAS.

Results

Milk production was unaffected by treatment ($p>0,05$), averaging 25.7 L/d. Milk protein was greater ($p<0,05$) in HSW comparing with LSW and CON (3.43%, 3.28% and 3.27% respectively). Milk fat, urea and lactose were not modified by seaweed inclusion ($p>0.05$) averaging 3.0%, 356 mmol/L and 4.7%, respectively. Solid not-fat in milk were higher in HSW treatment ($p<0.05$) compared to LSW and CON (8.99, 8.64 and 8.76 kg/d, respectively). The ammonia volatilization (expressed as % of total N application) from urine were lower for LSW (16.7%), followed by HSW (17.7%) and Control (18.5%) showing a quadratic effect ($p<0.01$). Dung ammonia volatilization did not differ between treatments ($p>0,05$) averaging 3.6%

Conclusions

The inclusion of *Macrocystis pyrifera* in diet of grazing dairy cows did not affect milk production, but increased milk protein as high inclusion rate. *Macrocystis pyrifera* reduced quadratically the ammonia emission from urine, but didn't affect the dung ammonia emissions. These results indicate that supplementation with M. pyrifera did not modify milk production and slightly reduces urine ammonia emissions in grazing dairy cows.

1729 - The impact of ambient temperature and relative humidity on the milk urea nitrogen levels of Holstein cows.

Author: Takula Tshuma, Dietmar Holm, Geoffrey Fosgate, Edward Webb, Corlia Swanepoel

Objectives

The research explored how ambient temperature and relative humidity affect the concentration of milk urea nitrogen (MUN) in Holstein cattle.

Material and methods

During the study period, data were collected from 161 Holstein cows with an age range of 642 to 2696 days and parity ranging from 1 to 5. These cows were intensively managed in a single group and fed a total mixed ration (TMR) three times daily. The study encompassed three distinct lactation rations (Diet 1, 2, and 3) with unknown compositions and analyses, administered during different periods. Composite milk samples (n = 2174) were collected every five weeks from each cow for the determination of milk urea nitrogen (MUN) concentration and other metabolites.

The milk sampling process, conducted during the afternoon milking session, involved aseptic collection into 35ml plastic tubes containing the preservative bronopol. The samples were transported to the laboratory within 6 hours. MUN concentration was determined through infrared spectroscopy, and somatic cell counts (SCC) were concurrently analyzed by the same machine. Data, including the age of cows, days in milk (DIM), milk production in the past 24 hours, MUN concentration, parity, and SCC for each test date, were obtained from the AFIfarm system.

Meteorological data, comprising daily minimum and maximum temperature and daily relative humidity measured at 08h00, 14h00, and 20h00, were obtained from the nearest South African Weather Services (SAWS) station. The temperature humidity index (THI) was calculated using the formula $THI = 0.8T + RH/100(T-14.4) + 46.4$, where T is ambient temperature in °C and RH is relative humidity. Minimum, average, and maximum THI were calculated for each milk sampling day and categorized into three heat stress levels: Low ($THI \leq 72$), Moderate ($72 < THI < 78$), and Severe ($THI \geq 78$).

Statistical analyses involved assessing the normality of continuous data, with non-normal variables being log-transformed. Scatter plots were used to visualize relationships before applying a linear mixed-effects model with a random effect for cow identification. Covariates such as age, DIM, heat stress level, ration, milk conductivity, milk production, parity, and somatic cell count were evaluated as main effects, along with the interaction term between relative humidity and temperature. The contribution of each variable to MUN concentration variation was determined using a general linear model.

All analyses were conducted using SPSS (IBM SPSS Statistics Version 23), with significance set at $P < 0.05$.

Results

During the study, a total of 2,175 composite milk samples were collected from 161 cows. The cows exhibited an average daily milk production of 31.41 ± 7.41 kg, with a mean milk urea nitrogen (MUN) concentration of 13.67 mg/dL and a somatic cell count (SCC) of 408.08 ± 1021.89 per ml. The environmental conditions were characterized by an

average temperature of 26.09 ± 5.05 ºC and humidity of 64.45 ± 19.12%. The age of cows at the time of sampling ranged from 21 to 90 months. Notably, 54% of the cows survived to the second parity, with 27%, 7%, and 2% reaching the third, fourth, and fifth parities, respectively.

Eight variables were retained in the regression model. Maximum temperature and maximum humidity on the day of sampling demonstrated a positive association with MUN concentration ($P < 0.001$), while the interaction term between these variables exhibited a negative association ($P < 0.001$). Heat stress ($P = 0.002$), milk yield ($P = 0.011$), days in milk (DIM) ($P < 0.001$), and SCC ($P < 0.001$) were positively associated with MUN concentration, whereas ration showed a significant association ($P < 0.001$).

The variables contributing the most to the variability of MUN concentration in this study were ration (18.19%), temperature (14.26%), humidity (13.12%), and other unassessed factors (24.7%).

Conclusions

The study identified that the maximum daily ambient temperature and relative humidity significantly influenced the measured urea nitrogen concentration. Therefore, it is crucial to take these environmental factors into consideration when interpreting urea nitrogen concentration data for making inferences about the dietary management of cows. For herd managers who rely on past urea concentration measurements to identify cows at risk of poor reproductive performance, adjustments based on ambient temperature and relative humidity are recommended for accurate and meaningful data interpretation. This underscores the importance of accounting for environmental conditions in understanding and managing the nutritional aspects and reproductive performance of dairy cows.

1808 - RELATIONSHIP BETWEEN RUMINATION TIME AND METHANE PRODUCTION IN HOLSTEIN COWS

Author: María Denisse Montoya Flores, René Calderón Chagoya, José Luis Romano Muñoz

Objectives

The study aimed to assess the relationship between rumination time and the methane production of Holstein cows in an automatic milking system.

Material and methods

The study included 28 Holstein cows: 8 from first calving, 10 from second calving, and 10 from three or more calvings. Cows were monitored from 6 days after parturition until the completion of the 305-day lactation period. Cows received *ad libitum* a partial mixed ration according to the National Research Council (2001), consisting of corn silage, oat hay, sorghum straw, flaked corn, sugarcane molasses, soybean meal, canola meal, whole cottonseed, and a mineral premix. Chemical composition of the ration expressed in percentage of dry matter (DM) was: DM, 58.3%; crude protein (CP), 16.5%; neutral detergent fiber (NDF), 33.3%; acid detergent fiber (ADF), 22.3%; and net energy of lactation (NEL), 1.68 Mcal/kg. Additionally, a concentrate in the form of pellets, consisting of the aforementioned ingredients excluding the forages, was offered at milking time (DM: 91.1%; CP: 20.1%; NDF: 15.4%; ADF: 6.6%; and NEL: 1.78 Mcal/kg). Data on milk production (MP) was collected from automatic milking systems, while rumination time (RT) was measured using electronic loggers on neck collars (SCR®; SCR Engineers Ltd., Netanya, Israel). The RT was registered in the software for processing the electronic records (Data Flow software, SCR Engineers Ltd.). Methane emissions were daily recorded throughout the lactation period using a Guardian-NG-Infrared Gas Monitor® system (Guardian Plus, Edinburgh Instruments Ltd., Livingston, UK). Methane measurements were expressed as grams of methane per day (CH_4/d) and grams of methane per kg of milk produced (CH_4/milk). Methane emissions were calculated following the methodology described by Garnsworthy *et al*. (2012). RT was categorized into low (LR), medium (MR), and high (HR), using a ±0.5 standard deviation thresholds (±17.7 min) (Nkrumah *et al*., 2007). Statistical analyses were conducted using the GLM and CORR procedures of the Statistical Analysis System software and the effects were analyzed using Tukey´s test ($p < .05$).

Results

Differences in RT were observed among all three rumination groups ($p< 0.05$). The values found were as follows: cows in the HR group, 628±3.1 min/day; MR group, 585.1±2.2 min/day; and LR group, 543.0±3.2 min/day. No significant effect of the rumination group ($p> 0.05$) was observed for CH_4/d (337.6 g/d, 340.4 g/d, and 356.0 g/d, respectively for HR, MR, and LR) or MP (42.3 kg/d, 40.2 kg/d, and 39.3 kg/d, respectively for HR, MR y LR). RT had a significant effect on CH_4/milk ($p< 0.05$). Cows from the HR group produced less CH_4/milk (8.5±0.3 g/kg) compared to cows from the LR group (9.9±0.3 g/kg). These results are consistent with those reported by Mikuła *et al*. (2022), indicating that RT promotes a decrease in CH_4/milk. RT showed a positive association with MP and a negative association with CH_4/milk, with correlation coefficients of r=0.24 (p=0.028) and -0.33 (p=0.003), respectively. Krpálková *et al*. (2022) found similar correlations for RT and MP.

Conclusions

Rumination time correlates positively to milk production and negatively to methane per kg of milk during the entire lactation of Holstein cows. However, these correlations between RT and MP, as well as between RT and CH_4/milk, were found to be low.

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SHORT COMUMUNICATIONS

1083 - The Virus Neutralization Test reminds us of the basics of bovine rotavirus immunisation

Author: Maxi HARZER, Luc DUREL , Audrey Brunet

Objectives

Group A Bovine Rotavirus (BRV) is a leading cause of neonatal diarrhea in cattle. The epidemiology is dominated in Europe by spatiotemporal differences in BRV strain distribution. Serotypes G6, G10, in combination with serotypes P[5],P[11], P[1] are the most frequently observed. In the general cattle population, outbreaks are frequent and, most often, unapparent. Therefore, the immune status of animals raised in commercial conditions has yet to be discovered. Several vaccines are marketed in Europe, formulated with inactivated viral particles, serotypes G6, P[1] (standard) or G6, P[5] (rare). Monovalent vaccination of pregnant dams is deemed to protect newborn calves, provided they are fed colostrum sufficiently shortly after birth. Commercial ELISA tests are sometimes used to assess the immune response in the animal but hardly predict the level of protection induced. Under these conditions, the public wonders about choosing the most suitable vaccine. This study recalls the interest of virus neutralization tests (VNT) in correctly interpreting immunization against BRV.

Material and methods

The design of a field trial was to depict the immune response to two commercial BRV vaccines for pregnant cows. Serological screening (Monoscreen AbELISA Rotavirus Bovin, BioX, Belgium) was carried out on approximately 160 pregnant heifers to isolate a subpopulation of animals seronegative for the BRV. Some weeks later, researchers picked up 43 animals from this subpopulation, and randomly vaccine them with BOVILIS®ROTAVEC®CORONA (RC, n=11, strain G6P[5]) or BOVISAN®DIAR (BS, n=22, G6P[1]), or left unvaccinated (CTRL, n=10). Blood samples were taken on the day of vaccination (D0) to perform an ELISA test. At D28 for ELISA again and a virus neutralization test (VNT) against BRV strains serotypes G5P[7] (porcine strain), G6P[1], G10P[11] and G6P[5]. The collection of colostrum at calving and performed a VNT.

Results

Before the trial, the serological status of the initial population was relatively homogenous, with median %inhibition >25 (ELISA). The animals selected constituted a cluster of virtually seronegative individuals. After a few weeks (D0), their serological status had changed, with most animals now being seropositive (median %inhibition >60). Twenty-eight days after vaccination, all animals were highly seropositive (>95%), including the CTRL group (>80%).

VNT performed on D28 revealed that CTRL animals were slightly positive to G5P[7] and G6P[5], and positive to G6P[1] and G6P[10]. In vaccinated animals, both vaccines elicited a strong immune response against G6P[1] with significantly higher ($P<0.05$) antibody titers than in control animals (Median=10.1, 95%CI[9.6;10.6], 9.9, [9.0;10.9], and 6.8, [5.1;8.6], for BS, RC and CTRL animals, respectively). Vaccination did not increase antibody titers against G6P[5], which remained significantly lower ($P<0.05$) than the other three serotypes tested. At the same time, there was a slight increase in titers against G5P[7] and G10P[11] (n.s.).

In colostrum, the VNT measured antibody titers higher than those measured in serum, particularly for G5P[7], G10P[11], and G6P[1] to a lesser extent. The median antibody titers were especially high for the three serotypes and significantly lower ($P<0.05$) for G6P[5]. For each of the serotypes, we did not detected any significant difference between control animals and vaccinated animals

Conclusions

This field study reminds us of a critical point of vaccination against BRV that is too frequently neglected by veterinarians and ignored by farmers: vaccination first induces the production of antibodies against serotypes with which the animal has had experience. In this case study, animals likely underwent an uncommon infection with G6P[1]. They had been exposed sooner to G5P[7] from swine-origin and G10P[11]. Vaccination has strengthened the immune status against these three serotypes, and the colostrum's potential to transfer this status to the calf was excellent. Conversely, animals never had experience with G6P[5]. The immune response in naive animals did not compare with the response of naturally primed animals.

This work confirms the low informative value of the ELISA test for the assessment of the immune status of commercial cattle. Using VNT to investigate apparent vaccination failure should be taken into consideration

1123 - Immunogenicity of two vaccines against neonatal calf diarrhea. European Pharmacopoeia standpoint

Author: Alexandra CERVANTES, Luc DUREL , Fabien SENSEBY, Victoire TRIMAUD, Audrey Brunet

Objectives

Neonatal calf diarrhea (NCD) remains a significant economic concern in Europe. Besides *Cryptosporidium parvum*, several pathogens can still cause outbreaks of NCD, the Bovine Rotavirus (RVA), the Bovine Coronavirus (BCoV), and *E. coli* K99 (ECK99). The industry markets several vaccines against these last three pathogens, frequently combined. A priori, and considering the conditions for obtaining marketing authorizations (MA), all these vaccines are effective. However, post-MA research sometimes reveals surprising and often contradictory results, consequences of unknown epidemiological situations and immune status, and unsuitable analytical methods. This work proposes using a rodent model based on the Pharmacopoeia Europaea (PhEUR) guidelines to evaluate the typical immune response elicited by two commercial vaccines.

Material and methods

The PhEUR vaccine potency assay monographs #0961 (neonatal colibacillosis of ruminants), #1952 (bovine viral diarrhea), and #1953 (Coronavirus diarrhea of calves) grounded the development of the rabbit model. Twenty-three female New Zealand White rabbits, 2.0-2.7 kg BW, SPF, were divided into two vaccinated groups (n=2x10) and a control group (CTRL, 3 animals). Vaccinated rabbits received two subcutaneous injections at a 2-week interval, on D0 and D14, of 2 mL of BOVILIS ROTAVEC®CORONA (RC) or BOVIGEN®SCOUR (BS). Animals were bled at D0, D14, and D28. Then sera were prepared and assayed with three commercial ELISA test kits (Monoscreen AbELISA Bovine Rotavirus, Bovine Coronavirus, and E. coli F5, all from BioX, Belgium). Sera were diluted when needed to estimate antibody content (ECK99, ½; RVA, 1⁄20; BCoV, 1⁄100). Optical density (OD) of transmitted light through the mixture was measured at 450 nm wavelength, and expressed as % inhibition=$[(OD_{negative}-OD_{sample})/OD_{negative}]x100$. The threshold for positivity was set at 20%.

Results

Clinical monitoring of the animals (weight, general health condition) revealed no changes in the animals. All control animals remained seronegative for all three markers. As regards the antibodies against ECK99, the mean % inhibition was greater than 50 from D14 for the RC group and stayed at this level until D28. In contrast, animals in the BS group remained seronegative until D14 before converging with those in the other group at D28. If we are interested in BCoV, the antibodies were noticeable from D14 in the RC group (>60%) before declining sharply at D28. In the BS group, antibodies were barely detectable at D14. However, at D28, the animals were strongly seropositive (86%). The RC group animals never produced antibodies against RVA, whereas the BS group animals were already clearly positive at D14 (40%) and strongly positive at D28 (>70%).

Conclusions

The rabbit model allows a study of the immunogenic potency of different vaccines under standardized conditions that respect the recommendations of the PhEUR for similar products. The responses observed in the model do not predict the protection obtained in the newborn of the target species (cattle). However, BS initiates a satisfactory immune response to the three antigens, even if the onset of this response is sometimes slow. RC triggers a solid and quick response against ECK99, but the responses to RVA and BCoV must be revised. The veterinary pharmaceutical industry essentially decides on the test model for vaccines, and there is no official model for BRV vaccines in the EU. Developing a single model for evaluating the immune response to vaccines against neonatal diarrhea in calves should be encouraged.

1263 - A case report of Campylorrhinia congenita in a Holstein calf

Author: Ana Carolina Martins Bock, Rüdiger Daniel Ollhoff , Juliana Melo Pankratz, Diógenes Adriano Duarte Santana, Eduardo Fernandes Teixeira

Objectives

Report a case of *Campylorrhinia congenita* in a Holstein calf at the Gralha Azul farm hospital, Pontifícia Universidade Católica do Paraná (PUCPR), located in the municipality of Fazenda Rio Grande, state of Paraná, southern Brazil.

Material and methods

The university hospital received the donation of the calf because the prior owner didn't like its appearance. The calf was delivered in an eutocic parturition, had a development in the average of the herd and came to the hospital at the age of ten months, weighing 103 kg. At examination exhibited an alert demeanor during the general and specific physical examination. The body condition score was 3 (1-chachetic to 5-obese), with a temperature of 38.1ºC. The feed intake was regular and showed no signs of difficulty. Mucous membranes were pink, and vital signs included a heart rate of 85 bpm with regular rhythm, and a respiratory rate of 24 bpm, costo-abdominal in type with superficial amplitude. In the specific clinical examination of the face, a facial deformity was observed twisting the last third of the face to the right side, affecting the maxilla, nasal sinuses, and mandible, with flattening of the left nasal region. The airflow during expiration was tested, revealing a smaller but more intense airflow in the left nostril when compared to the right nostril. A small quantity of sero-mucous nasal discharge was present in both nostrils. It was possible to hear the heifer snoring slightly due to nasal stenosis. Percussion of choanas and sinuses were inconspicuous. As a complementary examination, a dorsoventral projection X-ray of the mandible and maxilla was also conducted, revealing also a campylognatia, with deviation of maxilla and choanas, but allowing the exclusion of trauma due to the absence of fractures and bone callus.

Results

The clinical examination confirmed the diagnosis of *Campylorrhinia congenita* associated with campylognatia. The condition, although rare, is known in different species of animals, including horses. In cattle it seems to be predominantly diagnosed in Holstein. The calf after donation has been incorporated to the university herd and is developing without any incidents.

Conclusions

This is the first report of *Campylorrhinia congenita* in Brazil. Specific examinations on the heifer provided a detailed understanding of the extent of the malformation, indicating that the etiology of the condition is not associated with trauma. The major consequences related to this abnormality could be breathing difficulty, or difficulty in food apprehension, later however not observed in our case. The cause of this malformation is still unknown.

1301 - Granulomatous lymphadenitis as a possible etiology of vagal indigestion syndrome in cattle

Author: Rodrigo González López, Arturo F. Olguín y Bernal, Mario A. Bedolla Alva, Ulises J. Bautista Pérez, Angélica Ruiz Romero

Objectives

The average prevalence of bovine tuberculosis in Mexico is estimated in 16% in dairy cattle and just under 1% in beef cattle, which represents a significant number of affected animals. A according to data from the Agrifood and Fisheries Information Service (SIAP), in 2022 estimated a population of 2.68 million dairy cattle, out of a national inventory of 36.3 million.

Material and methods

In the present case description, the history of the disease is described and the lesions found in the necropsy and histopathological examination were related to the pathogenesis of a 5-year-old female Holstein Friesian cow with a clinical history of chronic recurrent gaseous bloat. This bovine was housed in a dairy with a history of bovine tuberculosis, located in the state of Hidalgo, Mexico. In the interrogatory, was reported that the cow died during the night.

Results

At necropsy, the relevant macroscopic findings were: distended abdomen, subcutaneous tissue of the ventral region of the mammary gland and pelvic limbs distended by edema, heart with rounded shape and mucinous appearance of the coronary fat, mucous membrane of trachea and main bronchi with multiple ecchymosis and suffusions, lungs congested and distended by edema, rumen distended by gas accumulation; enlarged liver and parenchyma with "nutmeg" appearance; right retropharyngeal and mediastinal lymph nodes increased in size and firm consistency, and its parenchyma showed some foci with caseous material.

In the histopathological examination, sections of mediastinal lymph node were revised, and stained with hematoxylin-eosin, showing extensive areas of caseous necrosis, and a strong pink coloration in the center indicating calcium deposits; multifocal areas with abundant Langhans type giant cells, and epithelioid cells were also observed; the Ziehl-Neelsen stain showed the presence of acid-fast bacilli.

Conclusions

Vagal indigestion syndrome is a functional or mechanical condition in the cranial nerve "X" (vagus nerve), of multifactorial origin of chronic course, affecting the motility of the gastrointestinal tract. The main causes of vagal indigestion syndrome in cattle are traumatic reticulitis, tuberculosis and lymphosarcoma. The importance of this syndrome is due to the alteration in gastric motility, which triggers a gas accumulation in the rumen and notable distention of the left paralumbar fossa, which can be intermittent or continuous, but tends to be progressive. Excessive rumen activity (hypermotility), hypomotility or complete atony may be found.

Based on the macroscopic and microscopic findings, this animal died due to gaseous tympanism, associated to the indirect mechanical compression of the vagus nerve by the mediastinal lymph nodes; the lesion in these organs suggests infection by *Mycobacterium bovis* that triggered a type 1 vagal indigestion syndrome, due to failure in the eructation with the subsequent inability to eliminate the gas promoting its

accumulation and distension of the rumen. The gaseous bloat originated a disorder of the circulating blood volume of the abdominal viscera, compressing the blood and lymphatic vessels, as well as the caudal vena cava, which was reflected by an increase in the hydrostatic pressure, which triggered right ventricular dilatation, ascites and ventral edema. Chronic passive congestion of the liver developed as a consequence of the blood volume disturbance caused by compression of the caudal vena cava due to the distended rumen.

The diagnosis of vagal indigestion is performed on the basis of clinical history, clinical signs, on general physical examination, and necropsy findings. However, the diagnosis is incomplete until the primary cause of vagus nerve dysfunction is determined.

Granulomatous lymphadenitis of the retropharyngeal and mediastinal lymph nodes from the observed tuberculosis occurs as a consequence of inhalation or ingestion of *Mycobacterium bovis*. Bovine tuberculosis is a chronic infectious-contagious disease whose etiological agent is bacteria of the genus *Mycobacterium bovis*, which are closely related to *Mycobacterium tuberculosis*, which also affects cattle. Tuberculosis is characterized by the formation of granulomas in various organs, and by a decrease in the physical and productive condition of cattle, and affects practically all mammals, including humans.

1337 - Cattle intoxication due to consumption of parota (Enterolobium cyclocarpum) in Mexico.

Author: Miguel Angel Quiroz Martínez, Julio Gamboa

Objectives

The aim of this word is to present a clinical case that occurred in a cattle ranch located in the north of the state of Veracruz, Mexico.

Where a group of cattle under grazing conditions was poisoned by the consumption of parota seeds also known as Guanacaste in this area.

Material and methods

Out of a group of 40 cattle of different ages, five of them suffered skin burns, two of which were severe. Most of the lesions occurred in extensive areas of the costoabdominal region and in the brisket area. The parota trees were located within the pasture where the cattle were grazing.

Results

Field diagnosis was made based on observation of the cowboy and the clinical signs. The skin was observed spotted and parchment-like, covered with yellowish secretions that detach in the form of plaques, dragging the hairs and leaving exposed areas completely bleeding and reddened. According to the literature, the type of photosensitization caused by parota is of hepatic origin due to damage to the hepatocytes, causing photodynamic compounds to accumulate in the skin, making it very sensitive to ultraviolet radiation, causing dermatitis of variable intensity. Once the diagnosis was made, the cattle were removed from direct sunlight and sheltered in the shade. The affected cattle were treated with hepatic protectors, vitamin A and antihistamines, and also with a moisturizing ointment. All of them responded favorably.

Photosensitization can have different causes, although there are two perfectly identified conditions, the first one is due to the consumption of plants that have compounds known as photodynamic that make the skin sensitive to solar ultraviolet light causing different degrees of burns. The second is because plants are consumed that have toxic principles that damage the hepatic parenchyma and cause it not to synthesize various compounds correctly. Among these compounds is phylloerothrin, which comes from the degradation of chlorophyll at the intestinal level, then passes to the liver where it is normally conjugated and eliminated by bile. When it is not processed by the liver, it accumulates in the skin and makes it sensitive to ultraviolet light, causing burns of different degrees, especially in depigmented areas of the skin or in animals with white coats. The consumption of parota has been recognized as a cause of several intoxications and it is known that it is capable of causing hepatogenic photosensitization.

Conclusions

This case is interesting due to the fact that the affected cattle are of zebu breeds, mainly Brahman, which are supposed to have a greater resistance to solar radiation because of their dark coat. In this case the intensity of the skin burns in the different animals was variable, in some cases they suffered first degree burns and others even second degree burns.

1386 - Biomarker analysis of milk from dairy cows with clinical mastitis

Author: Lorenzo Viora , Theo Pepler, Emily O'Reilly, Nicola Brady, Ruth Zadoks, David Eckersall

Objectives

Mastitis stands as a pervasive and economically impactful health issue among dairy cattle, primarily stemming from intramammary bacterial infections. In cases of clinical mastitis (CM), distinguishing between Gram-positive and Gram-negative bacterial causes or identifying culture-negative (no growth, NG) mastitis remains critically valuable. Notably, the efficacy of antimicrobial therapy in mild to moderate CM caused by Escherichia coli or other Gram-negative species is uncertain.

The identification and measurement of host-derived biomarkers within milk significantly enhance diagnostic capabilities. This methodology relies on leveraging host-derived biomarker proteins, thereby enhancing sensitivity and enabling swift on-farm point-of-care applications. The introduction of sophisticated platforms capable of multiplexing, digitalizing, and expediting results has notably bolstered this diagnostic approach.

Aims

This study aimed to assess the diagnostic potential of established mastitis biomarkers, either individually or in combination, to differentiate CM cases caused by Gram-positive or Gram-negative bacteria and to identify cases with no bacterial growth.

Material and methods

Seven dairy farms in Scotland participated, with milk samples collected and categorized based on CM severity. Standard laboratory techniques were employed for microbiological analysis, and the concentrations of established milk biomarkers (Haptoglobin - Hp, Milk Amyloid A - MMA, C-Reactive Protein - CRP, Lactoferrin - LF, α-Lactalbumin - LA, and Cathelicidin - CATHL) were determined using various immunoassays.

The biomarker outcomes (Hp, MMA, CCRP, LF, LA and CATHL) were utilized for comparing groups: the Gram-positive group against the combined Gram-negative and NG groups. Classification tree models (cross-validated) were constructed using these biomarkers to predict Gram-positive bacteria presence vs the rest. The resulting model's sensitivity, specificity, and overall classification accuracy were calculated.

Results

A total of 94 milk samples were collected from udder quarters displaying clinical mastitis (CM). Analysis revealed the presence of NG (n=21), Gram-negative (n=44), and Gram-positive (n=29) samples based on bacterial determination. Biomarker concentrations were significantly elevated in CM cases compared to that of healthy cows, regardless of culture outcomes.

NG samples exhibited lower Hp levels (P = 0.01) and higher LA concentrations (P = 0.01) in contrast to Gram-negative samples. Conversely, Gram-negative samples demonstrated higher CRP levels than Gram-positive counterparts (P = 0.03). However, no significant differences were observed for LF (P = 0.07), MAA (P = 0.54), or CATHL (P = 0.45) among the clinical mastitis sample groups.

The classification tree model highlighted specific biomarker concentration combinations associated with Gram-positive samples compared to the combined Gram-negative and NG samples: CRP < 9.5 (µg/ml), LF >= 325 (µg/ml), and MAA < 16 (µg/ml).

Conclusions

Despite individual biomarkers not differentiating between Gram-positive and Gram-negative mastitis, the combination of multiple biomarkers suggested the potential in achieving this discrimination. Such multiplexed analysis could hold promise as a comprehensive on-farm diagnostic tool.

1388 - Economic evaluation of a cull cow feedlot in the mexican tropics

Author: Claudia Nayeli Perez Jimenez, Eduardo Posadas Manzano, Carlos Antonio Lopez Diaz

Objectives

To carry out an economic and productive evaluation of a fattening system, under a semi-intensive system, of cull cows in the tropics, as well as to know the daily and total weight gain of the cows.

Prepare an economic analysis of the fattening flock and present proposals to improve technical and productive performance.

Material and methods

The work was carried out in the ranch "Rivera del Carmen" located in the south of the state of Veracruz, in the municipality of Las Choapas. For this study, 15 cows of different genotypes with an average weight of 365 kg at purchase were grazed on a 0.75 ha surface, which are grazed with insurgent grass (*Brachiaria brizantha*) and were offered concentrate in a manger. They had a 15-day period of adaptation to concentrate consumption, starting with 4.66 kg in two rations and later increasing it until they consumed 12.50 kg at the end of the fattening period, together with 2 bales of star saint sunday forage (*Cynodon nlemfuensis*) daily, which were only given for 55 days. An initial investment of $181,774 was made, with a total average price per cow of $12,184.93 at $33.00 and $34.00 per kg. The fattening period had an average duration of 130 days.

The analysis is based on the 12 cows only, since two were pregnant and one died during fattening. Feeding costs were allocated to prorate the costs of the entire lot among the finished cows.

In the economic analysis of the fattening process, the process was divided into two parts: the purchase and sale operation and the fattening of the cattle.

Results

The cows achieved a daily weight gain of 960 grams with an average total gain of 123.75 kg, having a feed conversion of 10.58 kg of feed per kilo of meat produced.

With respect to the economic part of the sale of the kilograms purchased, it left a margin (taking into account the dead cow) of $25,386, which represents a profitability of 14.3 % on the purchase and sale.

During fattening, the cows produced a total of 1,440.45 kg (considering the 3% discount of the visceral content claimed by the buyer), which meant a value of $61,939.35 for the kilos sold. The total fattening costs were higher than the income obtained from the sale, so this process had a negative result amounting to $40,410.

The cost of production per kilogram of finished cow for feed was $70.01, which was 162 % more than the selling price.

The inclusion of the income from the sale and purchase and the fattening expenses allows us to estimate a total cost per kilogram of meat produced per cow of $48.24 and the cost of sale was $43.00, which represents a 12% loss, that is, $5.24 per kilogram produced, considering the cost of labor and paddock.

Adding the non-explicit expenses and the operating result together results in a net margin of minus $29,974.69, indicating that the investment was greater than the income.

Conclusions

In relation to the fattening process, losses were obtained that exceeded the benefits of the sale and purchase. According to the financial analysis and in terms of profitability, negative economic indicators were found. With the above results, it is suggested to plan the business, carry out a cost-benefit study, based on the costs of ingredients, livestock, other inputs and not to consider fattening during the winter and drought seasons.

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1454 - Determination of calcium in commercial products and its effect on blood in downer cows postpartum

Author: Ulises Jesús Bautista Pérez, Arturo Federico Olguin y Bernal, Rodrigo González López, Alejandra Vargas Vera, Ángel Daniel Torres Ruíz, René Rosiles Martínez, Luis Ernesto Gutiérrez Solís

Objectives

Calcium deficiencies are common in cows during the transition period, especially postpartum, due to the increased demand for energy and calcium to milk production, mainly in high-producing cows**.** Abnormal serum calcium concentrations can be classified as clinical at <5-6 mg/dL, and subclinical at 6-8 mg/dL.

During peripartum there is a demand for Ca by the fetus (5.3 g Ca/day) and colostrum production (13-18 g Ca), so that the requirements for Ca after delivery increases from 15 g Ca/day to more than 50 g Ca/day and causes a Ca imbalance due to the fact that the regulation mechanisms are inactive.

The homeostatic mechanisms to regulate calcium concentration are the increase of its intestinal absorption, the release of bone calcium into the blood and the reabsorption of calcium at the renal level. Delay in the response of these mechanisms is the most common cause of hypocalcemia.

Factors such as excessive Ca drain to colostrum, impaired intestinal Ca absorption, and slowness of Ca mobilization from bone tissue to maintain calcemia, are triggers for hypocalcemia to occur.

Clinical signs begin 24 to 48 h after delivery, consisting of reduced feed intake, ruminal, abomasal and intestinal hypomotility, incoordination and motor loss, dystocia, placental retention and prolapse due to reduced muscle contraction.

Treatment should be immediate, especially if the animal is prostrated, since the pressure exerted by the weight of the animal on the locomotor limbs can cause "Downer cow syndrome" in only 4 hours due to ischemia of muscles and nerves and subsequent necrosis.

One of the fastest way to restore Ca concentration is the IV administration of Ca salts. The molecules frequently found in the market are calcium borogluconate, calcium gluconate, calcium levulinate, among others.

The IV Ca dose should be 2 g Ca/100 kg PV and administered at a rate of 1 g/min to avoid producing arrhythmia and cardiac arrest. This treatment increase blood Ca for 4 hours.

The preventive method that is frequently used is the implementation of anionic diets 2-3 weeks before delivery, causing a controlled metabolic acidosis, which stimulates PTH to mobilize Ca from the bones to neutralize this acidity, in addition increasing its intestinal absorption.

Material and methods

Twenty samples of intravenous calcium solutions of different commercial brands were analyzed by atomic absorption spectrophotometry. The percentage of calcium present in each solution was considered. The samples were digested with sulfuric acid and analyzed. All the commercial solutions were found to have a real calcium deficit in relation to what they show on the label, finding on average a deficit of -70.4%, with a range of -27.3% to -98.4%.

On the other hand, blood calcium was analyzed in 4 cows (b-1, b-2, b-3 and b-4) with clinical hypocalcemia before and 2 hours after the application of an IV calcium drug

Results

The results of serum calcium concentration (m g/mL) before and after drug administration were for b-1 of 11.41 and 11.46, for b-2 of 12.31 and 12.44, for b-3 of 17.95 and 13.21, and for b-4 of 10.53 and 11.36 respectively. While the actual calcium concentration found by atomic absorption spectrometry of 4 different solutions (in g/mL) administered for b-1 was 0.061 (it most contain 2.16, deficit of 97.14%), for b-2 it was 0.065 (must contain 2.16, deficit of 96.98%), for b-3 it was 0.038 (must contain 2.42, deficit of 98.42%) and for b-4 it was 0.056 (must contain 2.08, deficit of 97.31%).

Conclusions

In these 4 clinical cases the cows stood up once the calcium solution was administered to treat hypocalcemia, however, all 4 cows presented normal calcium levels before its application and the 4 solutions analyzed presented a large deficit of real calcium, so there was no influence on a real hypocalcemia condition.

The 4 calcium solutions were also tested for Mg, P, Na and K, however, none of the drugs are reported to contain these minerals. Probably the 4 clinically laid cows suffered from some other mineral deficiency, reestablished after drug administration.

Generally, in field practice it is inferred that any cow lying down in the postpartum period has hypocalcemia, but other causes of downer cow syndrome should be considered.

1460 - Burn dermatitis in three bovines, clinical case resolution

Author: Rodrigo González López, Arturo F. Olguín y Bernal, Ulises J. Bautista Pérez, Alejandra Vargas Vera, Angel D. Torres Ruíz

Introduction

A burn is inflammation, destruction of integumentary structures, which can involve skin, subcutaneous and muscles. The lesions denature cellular proteins, compromising their metabolism and leading to cell death. Direct contact with fire can be one of the main causes of burns, the damage will depend on time of contact and temperature.

The alterations that can develop in animals with burns are hemolytic and respiratory. When there is an extensive burn, inflammatory mediators in the organism are released, causing a systemic inflammation where vascular permeability increases and consequent edema which leads to hypovolemia that generates a decrease in perfusion and oxygen supply to the tissues. On the other hand, respiratory tract injuries occur due to inhalation of hot air, developing edema and obstruction of the respiratory tract.

Material and methods

Clinical case

Three adult cows were presented with burns after a gas explosion, showing burns of 50-70 % of the body surface of different degrees, incluiding the face, ears, udder, (mainly the teats), in all of them. During the clinical evaluation, the cows showed dyspnea and manifestation of severe pain, condition that caused the cows to hardly move, impossibility to lie down and eat.

The cows did not present fever; heart rate, respiratory rate and ruminal movements were within normal ranges. Corneal ulcers were found in all 3 cows. Cows 1 and 2 presented dyspnea, however, in cow 1 it was more severe, being the only one that presented bloody crusts in both nostrils.

In cow 1 and 2 there were burns on the face, eyes, ears, thorax and abdomen, presenting intense reddening of the skin together with blisters, hair loss and ulcers in the affected areas. Cow 3 was the least affected externally, the lesions involved the face, ears and body surface.

Results

Treatment

For all three cases, corneal ulcers were treated locally with chloramphenicol, ciprofloxacin, EDTA and vitamin A based drops.

In cow 1, dexamethasone, diphenhydramine and enrofloxacin were administered parenterally for the first 24 hours, then the anti-inflammatory was replaced by flunixin meglumine for 5 days.

To reduce dyspnea, salbutamol was administered intranasally with a nebulizer, 20 shots, daily, gradually decreasing the applications until they were suspended after two weeks.

The burns were treated topically with aloe vera once a day and Epidermal Growth Factor (EGF) in liquid form every 4 hours. Aloe vera was discontinued due to skin dehydration, after one week of treatment. Water wipes were placed locally over the most severe burns, every 30 minutes.

After one week the skin started to crack and purulent material was detected. A bacterial culture was performed and *E. coli* and streptococci were found. Enrofloxacin and flunixin meglumine were resumed for 5 more days.

After two weeks, cow 1 was sacrificed due to the severity of the lesions and general condition. At necropsy, pulmonary edema, pulmonary congestion, slight edema of the meninges, 30 cm hematoma in the abdomen and five ruminal ulcers were found.
In Cow 2, EGF was used on the burns, and an ointment based enrofloxacin and moisturizers. After the first week of treatment, the pain decreased and a correct regeneration of the skin was observed.
Two weeks after the event, the dermis began to fall off in several areas in the form of plaques, exposing the hypodermis. On inspection of the lesions, purulent material and abnormal physiological constants were observed, so the initial scheme was applied again for 5 days. After one month, one third of the ears had fallen off due to the necrosis that had developed. Likewise, patches (based on gelatin, pectin and carboxymethylcellulose) were placed on the scapula, in the region of the shield and in the lumbar area. Polyvinylpyrrolidone was applied in the groin, armpits and mammary gland.
In cow 3, bee honey and EFG were used locally for three weeks. At the end of the treatment only colloidal silver was applied.

Conclusion

Cattle are the domestic species with the greatest skin thickness, which undoubtedly protected the cows from more severe burns. In both cases, the evolution was favorable, with a remarkable regeneration of the skin, however, hair growth is no longer visible.

1494 - A PRELIMINARY STUDY OF SAPONINS IN AZADIRACTA INDICA (NEEM) A TREE IN MEXICO

Author: Silvia Denise Peña Betancourt, Eduardo Posadas Manzano

Objectives

Azadiracta indica A. Juss, grows in several states of Mexico as Morelos. it is known in Mexico as the Neem tree. This tree can measure up to 15 m, and its foliage is evergreen with leaves simple and alternate. The bark of the trunk is dark. We can found it in South America as Quillaja saponaria that grows in Chile, where the mapuches since ancient times used the bark as soap, or Quillaja brasiliensis, in northeastern Argentina, eastern Paraguay, northern Uruguay and southern Brazil.

Since 1970, when the first extract with saponins was obtained, it was demostrate their capacity as an adjuvant in a vaccine for veterinary use.

Saponins are soluble in water and form foam, however they have a complex chemical structure from the group of triterpene glycosides. Its molecular weight is 1.99 KDa and it acts by generating pores in cell membranes, which is why it has high toxicity.

Material and methods

The plant material was collected in the state of Morelos at the end of the winter and early spring (February and March) of 2018. The sample of complete plant was sent to the botany laboratory and the Toxicology laboratory for the analysis of metabolites. the leaves and bark of Neem were separated for dehydration, in an oven at 60 ºC. An ethanolic extraction was carried out from 50 g in 200 ml of 96% alcohol, for five hours in a sholet instrument and a rotary evaporator at reduced pressure, to obtain a concentrated ethanolic extract that it was to save in amber container until their analysis. The presence metabolites were identified using techniques based on specific reactions of each compound. For the alkaloids, Dragendorff's reagent was used, with which intense brick red and yellow stains were observed. For saponins, the foam method was used, which consisted of manually stirring a fraction of the aqueous extract for 20 min and confirming its presence if the foam persists.

Results

The plant was identified as Azadirachta indica A. Juss. Qualitative tests on both extracts showed the presence of tannins, alkaloids, triterpenoids and saponins. A higher saponin content was observed in bark than in leaves (0.25% vs 0.01%) and a higher tannin content was observed in the leaf extract (5.43 mg mL vs 1.75 mg mL). However, the presence of 4 secondary metabolites in our study cannot ensure its reproducibility since these compounds are synthesized by plants for their protection from the environment such as insects and fungi, so it will depend on the place where the plant grows. On the other hand, since they are complex mixtures of saponins that are extracted with water and ethanol, their real quantification is not possible, since more than 50 have been identified from extracts. Therefore, isolating and identifying each saponin in the extract is important since knowing its structure can make synthetic saponins, with the aim of protecting forests and avoiding an environmental imbalance due to the felling of Neem trees for industrial or artisanal use.

Conclusions

It was established that the ethanolic extracts of Neem bark and leaves contain the same four secondary metabolites qualitatively identified as alkaloids, tannins, saponins and triterpenes.

The presence of saponins detected in the ethanolic extract of the bark of Mexican Neem was greater than in the leaves, while tannins were greater in the leaves.

1560 - Antemortem diagnosis of aortic-iliac thrombosis in a calf with bilateral hind limb paralysis: a case report

Author: Eunwoo Chun, Jiyeon Kim, Hyoeun Noh, Woojae Choi, Danil Kim, Younghye Ro

Objectives

Thrombosis is a severe illness that can occur in various animals. Although cattle are more likely to develop venous thrombi, occurrence of aortic thrombi does occur in rare cases. Despite the low prevalence of aortic thrombi, there has been few reports on aorta-iliac thrombosis in critically ill-calves[1]. This rare condition of aortic-iliac thrombosis is quite easy to be mistaken for traumatic injury to the spine or hind limbs as the clinical sign is acute onset of paralysis. According to the location of the thrombosis, onset of paralysis or flaccid paralysis can occur on one or both side of the hind legs. Since this condition is not easy to diagnose, early treatment is rare, often leading to antemortem diagnosis. This is a case report of a Korean Native calf (Hanwoo), found in a comatose state due to severe diarrhea, which was finally diagnosed with aortic-iliac artery thrombosis.

Material and methods

A 20-day-old calf raised with its mother was referred to Farm Animal Medical Teaching Hospital, Seoul National University for being recumbent and depressed. Dirty tail suggested that the calf had diarrhea and Rota virus was confirmed by rapid diagnostic kit for the diagnosis of diarrhea. The body temperature was 33.9 ℃, and blood gas analysis was conducted on site with the result of decreased pH and bicarbonate concentration, negative value of base excess, hyperkalemia, azotemia, and hypoglycemia. Treatment for hypothermia, dehydration, metabolic acidosis was performed on site and antibiotics (Enrofloxacin), non-steroidal anti-inflammatory drugs (Meloxicam) and H2 blocker (Cimetidine) to reduce the amount of acid produced by the cells in the lining of the stomach were administered. Fluid therapy for metabolic acidosis and dehydration continued for three days on site and the calf regained its suckling reflexes and electrolyte imbalance was corrected. Upon physical examination, there was no sign of fracture of the leg, but the calf showed inability to stand up. With the onset of anorexia, the calf was hospitalized for further diagnostic tests.

Results

When attempting to rise, it was unable to put weight on its hind limbs despite assistance. Both its hind limbs remained cold with no palpable femoral arteries. Deep and superficial sensation was not present on both hind limbs and the tail was flaccid but with slight presence of perineal reflexes. Patellar tendon reflexes were absent with severe muscle weakness on both leg, which suggested musculoskeletal disorder. No other neurological abnormalities were noted. To confirm spinal abnormalities, radiography test was performed. After sedation, a contrast-medium was injected into the spine and images were obtained immediately after the injection but nothing abnormal was detected. In blood analysis, thrombocytosis, increased aspartate transaminase and creatine kinase activities, and increased glucose and blood urea nitrogen were noted. Although the calf had been treated with antibiotics, necrosis of the hind legs was severe as dry gangrene was observable and all reflexes and deep pain sensation remained completely absent. Putting together the results of the diagnostic test, the calf's

symptoms suggested the occlusion of the aorta and other arteries. The prognosis was judged to be poor and the calf died on 14th day of admission. In clinical necropsy, a thrombus was found at the abdominal aortic bifurcation to bilateral external iliac arteries, completely blocking both external iliac arteries. The muscles of both hind legs were severely necrotic. Caudal lobe of the lung was slightly congestive. No other findings were noted in the other organs, including umbilical cord and heart.

Conclusions

Reports of aortic-iliac thrombosis are not common in bovine veterinary medicine, thus diagnosis to treatment are not widely known. When acute posterior paralysis is reported in cattle, aortic-iliac thrombosis should be included in the differential diagnosis (DDx) along with spinal cord disorder and musculoskeletal disease. Clinical findings, including loss of femoral artery pulse, cold hind led extremities and acute hind leg paralysis are common findings in aortic-iliac thrombosis in cattle.

Currently, there is no standard treatment for aortic-iliac thrombosis. Treatment with anticoagulants is widely used in cats with hypertrophic cardiomyopathy and these drugs could possibly be used in calves if early detection is possible.

1570 - Application of soft tissue surgical procedures in dairy cattle.

Author: Mario Augusto Reyes Alemán, Ander Yuren , Jesus Cuevas

Objectives

The objective of this work was to evaluate the feasibility of performing a surgical procedure in a high-producing dairy cattle that suffered a gastrocnemius muscle tendon tear.

Material and methods

Patient data:

- Animal number: 88-06.
- Age: 2 years old.
- Sex: Female.
- Special mark: Being the highest producer of the place, iron with her number on the right side.

Clinical record: It was reported to the MVZ in charge of the place that the cow woke up lying on the handling chute with a lesion on the right hind limb, the manager made the observation that it could have been caused by some boards or the same tubes of the chute, which suggests that she had suffered a fall in the place and possibly got trapped in it and thanks to the struggle to be able to untrap the cow, the wound was inflicted.

General Clinical Examination: The patient was found to be in a physiological state within normal parameters.

Specific examination of the Locomotor System: The patient at the time of the examination was prostrate, so only the integrity of the affected limb was evaluated at the level of the gastrocnemius muscle tendon (soft and bony tissues) involved in the area of the injury (metatarsal), determining the tear of this tendon, causing the patient to present claudication with characteristic gait or locomotion deficits which also results in hyperextension of the fetlock and the entire distal part of the limb as a result of the reduction or loss of palmar support. Diagnosing a deep partial laceration/tear of the deep digital flexor tendon caused by a mechanically foreign object.

Pre-surgical: The animal was sedated to be able to perform the surgery with 2% xylazine at a dose of 0.5 ml/100 kg of weight intravenously. Subsequently, the affected area was washed with running water and chlorhexidine. After this procedure, the surgical area was then treated with 2% tincture of iodine. Necrotic and very damaged tissues were removed, in the same way it was necessary to reactivate the tissues in order to have an optimal recovery, taking into account and respecting the anatomy and functionality of the limb and tissues involved.

Surgical technique: We proceeded to perform the Bunnell suture technique for the tendon union and continuous suture to perfectly join the edges, the skin was closed with separate stitches.

Postoperative: A rigid PVC stalk with wadding padding was placed, a fixed bandage was applied to immobilize the pelvic limb in its distal part.

Results

For post-surgical treatment an analgesia and fluid therapy protocol was applied where 20 ml of flunixin meglumine was administered intramuscularly, 10 ml of Dexamethasone by the same route, 500 ml of intravenous glucose serum. Antibiotic protocol to avoid

secondary infections, the patient was given penicillin G procaine and benzathine 100,000 IU at a dose of 1 ml/ 20 kg of weight for 5 days, 20 ml of flunixin every 12 hours for 5 days, 10 ml of Dexamethasone for 3 days and a vitamin shot of 500 ml of A,D,E orally on the 2nd day after surgery, After 12 days the antibiotherapy therapy was changed to Ceftiofur for another 6 days, in a dose of 13 ml, which allowed us to work the withdrawals adequately, the patient could reintegrate to the productive line on the 28th day after her injury and surgical intervention, on the 18th day the patient could stand up and her recovery from this point on was optimal and very progressive.

Conclusions

This type of surgical interventions are not very common in the day to day of the medical clinic in bovines, but being the patient a high production animal, it was decided to perform this type of surgery. This allowed us to evaluate the possibility of treating this type of lesions at field level, for its diagnosis and treatment, resulting in the quick reintegration of the patient to his zootechnical life and thus avoiding the discard of the animal for not receiving good veterinary care, which is currently very common in dairy herds in our country (Mexico).

1739 - AMELOBLASTIC FIBRO-ODONTOMA IN AN OX

Author: Jesús de Nazare Zavaleta Hernández

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Objectives

Odontogenic tumors excepting canine acanthomatous ameloblastoma and peripheral odontogenic fibroma are rare in veterinary medicine. They represent a diagnostic challenge because of their component complexity, common features and subtle differences between them. These tumors are classified according to the presence or absence of different types of mature and embryonal dental elements. Ameloblastic fibroma and its variant comprising hard tissues (ameloblastic fibroodontoma–AFO-) are the most frequent odontogenic neoplasms in the oral cavity of domestic and wild bovids.

Material and methods

Case: An eighteen month-old Charolais ox was presented with history of feeding difficulties during the last month displaying a slow growing and 9 cm long bulging oral mass located on the incisor region that replaced bilaterally I1, I2 and right I3. The teeth loss was interpreted by the owner as a normal dental replacement. A marginal resection of the tumor was made, and the surgical biopsy was submitted for histopathological examination.

Results

Grossly, it was multinodular, white-gray and hyaline, firm, with tiny cystic spaces. There were some tan, irregular hard areas that protruded through the gum that required decalcification. Microscopically, there was a partially circumscribed tumor composed of trabeculae, sheets and buds of odontogenic epithelium embedded in abundant loose amphophilic ectomesenchyme. Some epithelial buds resembled the enamel organ and were associated with layers of homogeneous basophilic to eosinophilic acellular matrix, interpreted as dentin or enamel. There were also trabeculae of basophilic matrix with lacunae and reverse lines, compatible with cementum or osteodentin. Some of these arrangements seemed to form primitive or disorganized dental structures. Mitotic figures were rare, with mild anisocytosis and anisokariosis. The differential diagnosis for this mixed histological pattern is the ameloblastic fibro-odontoma and a developing complex odontoma, whose differentiation is not always possible with histopathology alone.

Conclusions

Odontogenic tumors are a diagnostic challenge. Familiarity with dental development and its anatomic components is fundamental for their diagnosis. AFO is a rare neoplasm that has been described more frequently in bovines. Most of the cases lack follow-up in the long term making difficult to determine if AFO is a separate entity or a stage of development of an odontoma.

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1814 - REPRODUCTIVE INDICATORS IN A DUAL-PURPOSE CATTLE SYSTEM IN HUMID SUBTROPICAL CLIMATE AF(C)

Author: René Carlos Calderón-Robles, René Calderón-Chagoya, Ángel Ríos-Utrera, Carlos Hernández-López, Itzel Yarisa Lucero-Flores, Juvencio Lagunes-Lagunes

Objectives
This study aimed to assess the influence of environmental factors such as year, season, and parity number on reproductive characteristics including interpartum interval (IPI), age at first calving (AFC), age (AFB), and weight (WFB) at first breeding in a dual-purpose system with Holstein x Zebu cows in the humid subtropical region of Puebla.
Material and methods
Data were collected from the "El Paraíso de Ayotoxco" dual-purpose module, located in Puebla, Mexico, at 240 meters above sea level with humid subtropical climate Af(c). Reproductive management (heifers, dry cows, pregnant, and lactating) involved intensive rotational grazing on 100.5 hectares of insurgent grass (*Brachiaria brizantha*) and 54.5 hectares of native grass where other system animals graze. Throughout the critical season (November-April), each cow that initiated production received a daily ration of 15 to 20 kilograms of freshly chopped Japanese cane (*Saccharum sinense*). Additionally, lactating cows were provided with 2 kilograms of concentrate (containing 16% crude protein and 70% dry matter) per milking. In addition to grazing, the calves' diet consisted of the milk they consume during milking and from a quarter of the udder, as well as any residual milk until they reach 100 kilograms of live weight. After reaching this weight, they only consume support milk and residual milk, along with a daily ration of 750 grams of concentrate containing 18% crude protein. Weaned heifers grazed rotationally and consumed 1.5 kg/animal/day of the same concentrate. Subsequently, until the start of reproductive management, they grazed rotationally on pasture. All animals had access to a mineral mix and water *ad libitum*. Mechanical milking was conducted twice daily, with the presence of the calf to stimulate milk ejection. Reproductive management consisted of continuous mating. Heat detection was performed during milking, and rectal palpation was conducted every three months to assess uterine-ovarian condition. Heifers in reproductive management were also subjected to continuous mating but without heat detection. The studied traits included IPI, AFC, AFB, and WFB. These traits were analyzed using the SAS GLM procedure, with fixed effects including year, season, and calving number.
Results
A total of 870 IPI were measured, with a duration of 488 days. IPI was affected ($P<0.05$) by calving number, being more prolonged and significantly different in first-calving cows (535±7.6 days). In second-calving cows, the IPI (497±8.3 days) differed from those with four or more calvings, but not from third-calving cows (482±9.5 days), although these did not differ from those with more calvings. Reproductive traits varied significantly by calving year for all studied parameters. Calving season only affected AFB, with females calving during the rainy season being younger than those during the dry season, but both groups were not different from those calving during the cold season. Although AFC is correlated with AFB, it did not differ between seasons.
Conclusions
In conclusion, reproductive indicators IPI and AFC are suitable for tropical conditions, especially when the system's profitability is adequate and there is potential to enhance reproductive performance.

1815 - PRODUCTIVE ANALYSIS OF CATTLE IN A DUAL-PURPOSE SYSTEM IN HUMID SUBTROPICAL CLIMATE Af(c)

Author: René Carlos Calderón-Robles, René Calderón-Chagoya, Carlos Hernández-López, Ángel Ríos-Utrera, Juvencio Lagunes-Lagunes

Objectives

The objective was to assess the effects of environmental factors such as year, season, and calving number on milk production per lactation (MPL), milk per day (MD), milk per interpartum day (MIPD), lactation length (LL) of Holstein x Zebu and Brown Swiss x Zebu cows in the humid subtropical region of Puebla, Mexico.

Material and methods

Information from 2010 to 2021 from the dual-purpose production unit "El Paraíso de Ayotoxco" was utilized. This unit is situated at an altitude of 240 meters above sea level in Ayotoxco de Guerrero, Puebla, and experiences a humid subtropical climate classified as Af(c). Cows were managed through intensive rotational grazing on 72 hectares of Insurgent grass (*Brachiaria brizantha*). Throughout the critical season, each female received a daily ration of 15 to 20 kilograms of freshly chopped Japanese cane (*Saccharum sinense*). Additionally, lactating cows were provided with 2 kilograms of concentrate (16% of crude protein and 70% total digestible nutrients) during each milking. All females received a mineral mix and had access to water *ad libitum*. Cows started milking four days after calving. Milking procedure was assisted by the presence of the calf to stimulate milk letdown. The reproductive management of the cows involved continuous mating. The traits studied were: MPL, MD, MIPD and LL. These traits were analyzed using the GLM procedure in the SAS program. The fixed effects included in the final model were: year, season, and calving number. Sequential analyses were conducted for each trait to determine the final models, removing non-significant interactions from the preliminary model at a significance level of $P<0.05$.

Results

A total of 934 lactations were evaluated, with an average LL of 339.65 ± 94.75 days and MPL of 3392.56 ± 1185.94 kg, as well as MD and MIPD of 9.94 ± 1.99 and 7.43 ± 2.36 kg, respectively. According to preliminary study results, LL remained the same, while the rest of the traits were lower (MPL =2905, MD =8.5, and MIPD =6.6 kg). This improvement is attributed to the increased adoption of technology developed by the National Institute for Forestry, Agricultural and Livestock Research of Mexico (INIFAP) in the demonstration module, leading to enhanced productivity indicators. The year and calving number were significant in all models, while calving season was only significant for MIPD. MPL, MD, and MIPD were lower in first-calving cows and gradually increased with each subsequent calving. Cows with more than three calvings showed the best performance. LL decreased, with the lowest observed in cows with more than five calvings. In the effect of calving year, it is observed that MD and MIPD have increased over the years, while LL and MPL tend to vary from one year to another, but without negatively affecting MD and MIPD. Cows that calved during the rainy season showed a higher ($P<0.05$) MIPD (7.64 kg) compared to those calving during the cold (7.34 kg) and dry (6.92 kg) seasons.

Conclusions

In conclusion, calving year and calving number significantly influenced all variables evaluated. Calving season only affected MIPD. The effect of lactation number indicates that first-lactation cows had lower MPL, MD, and MIPD, while cows with more than three lactations showed the best performance in these traits. The mean values of MPL, MD, MIPD, and LL in the validation module "El Paraíso" were: 3392.56 kg, 9.94 kg, 7.43 kg, and 339.65 days, respectively, surpassing the production obtained in the initial years due to the incorporation of technologies that had not yet been utilized.

EDUCATION IN RUMINANT VETERINARY MEDICINE AND PRODUCTION

1134 - Comparison of two recombinant bovine somatotropin products on milk yield and metabolic parameters

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Objectives
The study aimed to compare the efficiency of two commercial recombinant bovine somatotropin (rbST) products on the milk yield and metabolic parameters of mid-lactation Holstein cows.
Material and methods
A total of eighteen cows were randomly allocated to two treatment groups to be treated with two different injectable rbST-based products. The “rbST-Fast” group received a formulation of rbST containing lecithin and vitamin E (Boostin®, MSD Saúde Animal, SP, Brazil). The “rbST-Slow” group received a formulation of rbST containing sesame oil and zinc (Lactotropin®, Agener União Saúde Animal, SP, Brazil). Animals in each treatment group were treated subcutaneously with their respective rbST product at a dose of 500 mg of rbST every 14 days, resulting in a total of 5 administrations throughout the course of the study. Blood samples were collected at each cycle to assess metabolic markers, such as non-esterified fatty acids (NEFA). Milk yields were measured daily using the DelPro™ software (DeLaval®). Milk samples were collected weekly to analyze milk composition.
Results
The “rbST-Fast” group had a significantly higher milk production (p = 0.03) than the “rbST-Slow” group. In addition, cows in the “rbST-Fast” group had significantly higher (p < 0.01) serum concentrations of NEFA but lower (p = 0.09) serum glucose values than the “rbST-Slow” group. Regarding milk components, the “rbST-Fast” group had higher lactose content (p = 0.05) than the “rbST-Slow” group.
Conclusions
Cows in the “rbST-Fast” group showed significantly higher milk yield, serum NEFA and milk lactose concentrations as compared to the cows in the “rbST-Slow” group.

1248 - Ambulatory production animal practice: how is travel time used for student teaching and learning?

Author: Sarah Wood , Rebecca Vallis, Katie Bonefaas, Sarah Baillie

Objectives
Production animal veterinary practice, by its very nature, includes time spent travelling between visits throughout the working day. In the UK, veterinary students must undertake 26 weeks clinical extra-mural studies (CEMS) during the clinical part of their veterinary degree and many will choose to spend a proportion of this time in farm animal ambulatory practice. Additionally, in the final year of the programme veterinary students undertake placements in first opinion practice, commonly termed intra-mural studies (IMS). For students on both types of placements this time travelling with the practitioner makes up part of their experience and education. Despite travel time accounting for a proportion of the working day, what goes on during this period is an area previously unexplored in the literature surrounding training of veterinary students and preparation for clinical practice.
Material and methods
In the first part of this study, we used a quantitative approach to identify the number of hours students spend travelling with practitioners. Students at Bristol University undertaking CEMS in production animal practice recorded the number of hours spent in the vehicle with practitioners each day. Secondly, we used focus groups to explore the experiences of practitioners and students in depth. The aim of the focus groups was to identify how this time travelling together in the car to and from farm visits is currently used; what good teaching practice exists, what challenges are encountered, how these are managed or could be overcome and what support may be needed to use this time most effectively.
Results
Student reported data revealed that on average just over 2 hours per day is spent travelling with practitioners between visits. Thematic analysis of the focus group data demonstrated how time is typically used. Practitioners presented common topics that were used to stimulate discussion; these related to preparation for, or debriefing of, the work encountered on the visit and commonly identified knowledge gaps for students at different stages of their studies. Practitioners spoke of their approach to enabling what they perceive to be an effective learning environment, as well as challenges that exist in engaging students in learning. Students identified the importance of a positive and encouraging learning environment and questioning style. They voiced the benefit of prior awareness of planned work, and thus an ability to prepare and make good use of discussion time to and from the visits. Both groups saw the benefits of time spent discussing non-clinical topics; for example pastoral and career support.
Conclusions
Time students and practitioners spend travelling to and from visits is unique to ambulatory practice. It amounts to a considerable portion of the working day and represents a valuable opportunity for discussion. The outcomes of this study can be used to aid practitioners in using the time as effectively as possible to support and enhance student learning.

1295 - Effects of oral administration of heat-treated Lactobacillus sakei HS-1 on peripheral blood lymphocyte counts and leukocyte subpopulations in newborn Holstein cattle

Author: Atsushi Kimura, Yuma Nagao, Toshihiro Ichijo

Objectives

Newborn calves are low immunitiy and are susceptible to diseases such as diarrhea, so probiotics and prebiotics are commonly administered because these supplements directly act on intestinal flora. Recently, biogenics that stimulate intestinal immunity without affecting the intestinal flora have been of great interest. Sasazaki *et al*. reported that heat-treated *Lactobacillus sakei* HS-1 (HS-1) administered to Japanese black cattle effectively suppressed diarrhea and shortened the treatment period. However, HS-1's effect on immune function in calves remains unclear. In this study, we evaluated the effects of oral HS-1 therapy on immune function in newborn Holstein calves, specifically changes in peripheral blood leukocyte subpopulations.

Material and methods

This study was approved by the Iwate University Animal Experiment Committee (approval number A202327). Twelve Holstein calves from Iwate University and a nearby dairy farm were randomly enrolled in either the control or trial group. Both groups were administered the same feed. Base on described by Sasazaki *et al*., in trial group, 1% milk replacer`s weight of heat-sterilized HS-1 (Lactobacillus sakei HS-1; counts of HS-1: 10^7 / g; Miwa-Seiyaku, Tokyo) was added to milk replacer twice daily from 3 to 24 days after birth. Blood and feces were collected on the 1st, 7th, and 21st day of treatment. Fecal bacterial counts, blood biochemical properties, and peripheral blood leukocyte subpopulations were quantified. Peripheral blood leukocyte subpopulations were evaluated by flow cytometry, and the number of surface antigen-positive cells for WC1, CD21, CD14, CD3, CD4, CD8, and CD45 was calculated. Statistical analysis was performed using R (Ver. 4.3.1). The trial and control groups were compared by examining the normality of distribution, performing a two-way analysis of variance (ANOVA), and conducting Tukey's test for multiple comparisons. After estimating the distribution, two-way ANOVA and repeated-measures one-way ANOVA or Friedman test were performed to evaluate changes over time within the groups. Dunnett's test was performed to conduct multiple comparisons.

Results

No significant changes were observed in fecal bacterial counts or blood biochemical properties. However, a significant ($p < 0.05$) increase in the lymphocyte and $CD3^+$count was observed on days 7 and 21 after administration compared with day 1. $WC1^+$and $CD3^+CD45^-$ peripheral blood leukocyte subpopulations were significantly higher ($p < 0.05$) on day 21 of administration than on day 1 in the trial group. Although $CD4^+$, $CD4^+CD45^+$, and $CD4^+CD45^-$ increased with days in both groups, no significant differences were found.

Conclusions

HS-1 administration was associated with a significant increase in the $CD3^+$ cell counts on days 7 and 21 of treatment and $WC1^+$ cells on day 21. The increase in T and γδT cell

counts suggests that HS-1 administration affected cellular immune function. Furthermore, the increase in $CD3^{+}CD45^{-}$ cell counts on day 21 in the trial group indicates that HS-1 administration similarly stimulated immune function and increased the number of highly active T cells in the peripheral blood. Thus, future investigations on the detailed outcomes of cytokines like INF-ɣ, TNF-α, IL-2, IL-12, and IL-22 measurements are needed to elucidate the efficacy of HS-1 immune function-related mechanisms. Furthermore, in order to confirm clinical efficacy in newborn Holstein calves, detailed investigation is required to investigate the relationship between pathogens and HS-1 and the state of diarrhea.

Referrence

Sasazaki *et al*., (2020) Effects of dietary feed supplementation of heat-treated *Lactobacillus sakei* HS-1 on the health status, blood parameters, and fecal microbes of Japanese Black calves. J. Vet. Med. Sci., 80 (10): 1428-1435.

1376 - A blended learning intervention using an online conversation training tool improves veterinarians' communications with dairy producers

Author: Linda Dorrestein , Herman Barkema, Geert Vertenten, Caroline Ritter

Objectives
For dairy veterinarians, advice-oriented discussions with clients, specifically regarding preventive herd health procedures, can be challenging, as most practitioners have not been formally taught communication skills (CS). The objective was to determine the optimal communication education intervention to improve veterinarians' consultation abilities during herd health conversations.
Material and methods
Twenty-eight dairy veterinarians (4 women, 24 men, 27-62 years, 3-38 years of clinical practice), enrolled in a clustered-wedged randomized controlled trial for communication skills training interventions. They were allocated into 4 clusters: 1) control, no intervention; 2) webinar, 1.5-days online webinar in a small-group; 3) Veterinary DialogueTrainer (VDT), online training tool; and 4) blended, combining webinar and VDT. Pre- and post-intervention, participants audio recorded herd health management visits on dairies in Flanders, Belgium. Communication competency and consultation structure were assessed using a composite scoring system, based on Calgary-Cambridge Communication Guidelines, and specifically designed for this study. The recorded visit was evaluated on the presence of 10 essential dimensions of a veterinary consultation.
Results
Participants audio recorded 353 herd health visits on 218 dairy farms. Recordings averaged 1.5 hours and data extraction was possible for 83% of recordings. In preliminary assessments, most recordings from all clusters contained Creating rapport, Gathering information, and Conversation about cost if warranted. Cluster 3 (VDT) improved in their consultation structure in 2 of 10 dimensions, whereas Clusters 1 (Control) and 2 (Webinar) improved in 4 of 10 dimensions. However, Cluster 4 (blended learning intervention) was the most successful, with improvements in 8 of 10 dimensions.
Conclusions
A blended learning intervention was most effective in improving communication abilities of dairy veterinarians in herd health consultations. Further research is needed to assess whether communication training can lead to a sustainable change in veterinarians' communication behaviours.

1377 - Effects of communication training on dairy veterinarians' use of communication tools when gathering and providing information

Author: Linda Dorrestein , Caroline Ritter, Jannet de Jonge, Geert Vertenten, Herman Barkema

Objectives
As key information sources for dairy farmers, veterinarians have substantial impacts on herd health management decisions. Becoming more advice-oriented, specifically regarding preventive herd health procedures, is challenging for many dairy veterinarians, as most have limited formal communications training. A communication methodology that could help develop advising skills is Motivational Interviewing (MI); it promotes internal motivation of farmers to change behavior. The objective was to examine effects of various communication education interventions on dairy veterinarians regarding their ability to gather and provide information.
Material and methods
Twenty-eight dairy veterinarians (4 women, 24 men, 27-62 years, 3-38 years of clinical practice), enrolled in a clustered-wedged randomized controlled trial to receive communication skills training interventions. They were allocated into 4 clusters: 1) control, no intervention; 2) webinar, 1.5-days online webinar in a small-group; 3) Veterinary DialogueTrainer (VDT), online training tool; and 4) blended, combining webinar and VDT. Pre- and post-intervention, participants audio recorded herd health management visits on dairy farms in Flanders, Belgium. Communication competency and MI skills were assessed using a composite scoring system, based on the Motivational Interviewing Treatment Integrity (MITI) code and Calgary-Cambridge Communication Guidelines (CCCG), and specifically designed for this study. A 20-minute fragment of recorded heard health visits that focused on advising the farmer was assessed on MI and communication behaviours.
Results
Audio was recorded for 353 herd health visits on 218 farms, of which 48% had a 20-minute fragment suitable for assessment. Counts of 'Giving information' increased in all clusters. Number of open-ended questions asked increased in all clusters, with number of open-ended questions almost doubling in Clusters 2 and 4. Number of closed questions asked varied between clusters, with Clusters 1 and 3 asking less and Cluster 2 and 4 asking more closed questions post-intervention.
Conclusions
A possible explanation for increased open-ended questions and giving information across all clusters might be that participants were influenced by being audio recorded (Hawthorne effect). However, as Clusters 2 and 4 had the greatest increase in number of open questions, small group learning intervention was most effective in improving communication abilities related to gathering and providing formation. Further research is needed to assess whether this training translates into a sustainable change in dairy veterinarians' communication behaviours.

1383 - Who is best in showing added value as a bovine vet? Measuring the difference in consultancy communication skills between four European countries, a pilot study.

Author: jolanda jansen , Quintie Stoel, Tessa Plagis, Geert Vertenten, Klaas Okkinga

Objectives

The dairy industry faces many challenges over the past years. These challenges have direct impacts on the role of advisors and require the continuous development of knowledge and skills, especially on communication and behavioural change. Research has shown that communication skills play a vital part in delivering advice with impact, and yet many advisors are lacking in this department. Training communication skills can be very successful, provided that participants actively practice their newly acquired skills and receive feedback. Aim of this pilot study was to investigate the basic consultancy skills of bovine vets and the differences between countries.

Material and methods

The Veterinary DialogueTrainer (VDT) is an online computer game that trains, simulates, and measures realistic conversations between people. The VDT creates an opportunity to objectively measure performance on herd health consultancy conversations with a virtual dairy farmer and to compare skills between countries. VDT was applied in a communication skills training of bovine veterinarians in four countries between January 2022 and November 2023 : UK (n=68), France (n=14), Italy (n=12) and the Netherlands (n=25). Participants all received the same two computer game scenarios translated to their local language. Topics were: 1) taking the initiative to talk about coughing calves when visiting the farm for a caesarean and 2) as follow up having a structured and effective conversation about young stock health and disease prevention. Communication skills were measured on a 0-100% scale by answer choices throughout the conversation with the virtual farmer. Total score is based on parameters like maintaining a good relationship, clarifying needs and showing added value and were measured by underlying constructs like e.g. showing empathy, asking open ended questions and proactively explain the added value and offering services. Scores on the first attempt per scenario were compared between countries as well as their ultimate high scores, as players could play multiple times and improve their scores based on the received feedback as they finish the game.

Results

In all countries in simulation 1 players scored on average a total score of 25,7% on their first attempt and scored lowest on parameter ‘showing added value’ (12,8%). Common mistakes include jumping immediately to solutions without investigating farmers needs and wishes. In simulation 2 they scored in on average a total of 62,2% on first attempt, indicating initial improvement of skills after playing simulation 1. Players scored lowest in scenario 2 on building the relationship (59,6%), particularly in showing empathy and reflections on emotions and give opportunity for the farmer to add topics to the meetings’ agenda. In all countries players were able to achieve a total score of >75% on both scenario’s when playing multiple times, indicating a learning effect. Dutch veterinarians score best in communication skills on their first attempts for the first and second scenario (32,8% and 73,2%), Italian veterinarians score lowest on their first

attempts (19,6% and 48,9%) indicating a difference in herd health consultancy skills between countries.

Conclusions

Results suggest bovine vets' communication skills and their efficacy of their herd health advice in general could be optimized. There is a difference in performance between vets from different countries when presented similar situations. The VDT could be an effective method to simultaneously train and measure communication skills in various countries.

1508 - EVALUATION OF THE DIAGNOSTIC RELIABILITY OF A DIGITAL EXPERT DIAGNOSTIC TOOL (VETAID) FOR CATTLE DISORDERS

Author: RAPHAEL GUATTEO , François Schelcher, Quentin Petit, ME-Linh Phan, Yves Millemann, Audrey Ponard, Thibaut Lurier, Gérard Argenté

Objectives

Whether at the beginning of their career or when faced with atypical cases, veterinary practitioners may feel the need to confirm their hypothesis and ensure that none is overlooked. Having access to an accurate expert diagnostic tool would therefore be useful. To our knowledge, the only tool available in bovine medicine is the “Consultant” Cornell University website, which provides a list of differential diagnoses based on clinical signs, but does not consider the circumstances of onset or the animal’s characteristics, nor does it provide a hierarchy between hypotheses. In this context, the VETAID application (https://www.vetaid.net/) was created in 2020 by an experienced cattle veterinarian (Gérard Argenté) with the support of specialists from different French Veterinary Schools. Before distributing this tool more widely, it was considered judicious to challenge its performance in terms of diagnostic reliability.

The aim of this study, carried out jointly by the French Veterinary Schools was to assess the reliability of VETAID in correctly diagnosing cattle diseases considering the diagnosis made by an experienced veterinarian as the reference.

Material and methods

Three veterinary students recruited the cases during a six-month externship. Every day, they had to randomly recruit up to two cases (excluding dystocia, c-section and herd health visits) for which they had to conduct a clinical examination and develop hypotheses with their mentor. The mentor's first hypothesis was considered to be an expert diagnosis if he was confident in his hypothesis and/or confirmed by a specific test (laboratory, surgical or necropsy) or successful treatment. After employing VETAID in every case the vet students had to evaluate the hypotheses giving a score:

- Score of 5: The expert diagnosis is suggested in the list of diagnoses proposed by VETAID.
- Score of 4: At least one diagnosis suggested by VETAID is very close to the expert diagnosis, but not exact
- Score of 3: At least one of the diagnoses suggested by VETAID is closely related to the expert diagnosis and can help the practitioner in his reasoning
- Score 2: At least one of the diagnoses suggested by VETAID is related to the expert diagnosis but is not likely to help the practitioner in his reasoning.
- Score 1: No diagnostic proposal suggested by VETAID is close to the expert diagnosis

The percentage of cases with a score of 5 was then calculated to assess the diagnostic accuracy of VETAID for the following categories of diseases (>10 recruited cases): lameness, downer cow, digestive, mammary disorders, and miscellaneous. We also evaluated the rank of suggestion of the expert diagnosis for each type of intervention to see whether the correct diagnosis is more often suggested first or not. Finally, for cases without an expert diagnosis, we assessed whether VETAID's proposals were coherent and helped the veterinary surgeon in his diagnostic approach.

Results

There were 143 cases recruited during six months in three practices, 116 of which could benefit from expert diagnosis by the experienced vet of the practice; for the locomotor, digestive, diarrhoea, respiratory and digestive disorders, an expert diagnosis was made in respectively 82% (18/22), 84.31% (16/19), 66.37% (14 cases/21), 54.55% (6/11) and 62.50% (10/16) of the cases. On these 116 cases, the VETAID tool obtained a mean score of 4.63 out of 5 and provided an accurate diagnosis (score 5) for 96, i.e. almost 81% of correct diagnoses.

For each of the following syndromes, when an expert diagnosis was made, VETAID provided an accurate diagnosis in most cases: Locomotor 83% (15/18), downer cow 87% (14/16), digestive 93% (13/14), respiratory 60% (6/10), mammary 90% (9/10) and miscellaneous 68% (11/16). For cases with a score of 5, the VETAID application proposes expert diagnosis in the 1st position in 80% of cases as a mean. For the cases with uncertain hypotheses formulated by the mentor, VETAID provided relevant hypotheses. From a qualitative perspective, VETAID was considered by the vet student as a friendly user application.

Conclusions

This project would benefit from being carried out on a larger sample and a more diversified range of diseases. However, the VETAID application seems to be a promising tool to help students and practitioners in practice, particularly among the younger generation, who are well-versed in the use of digital tools.

1550 - Behavioural responses of sheep used for teaching veterinary undergraduates

Author: Sander Prins , Kathryn Ellis, Jayne Orr, Dorothy McKeegan

Objectives
This study aimed to fill a gap in knowledge regarding the behavioural responses of sheep during the teaching of clinical examinations with three objectives: Do sheep display stress behaviour during teaching and is there habituation to this behaviour? Is there a difference in maintenance behaviour before, during and after teaching? Which factors influence the stress behaviour?
Material and methods
For this study, six non-pregnant Easy-Care sheep were observed during their use in clinical examination classes for veterinary students. These classes took place once a week for five consecutive weeks. A total of 129 students examined the sheep during these classes. Sheep behaviour was video recorded before, during and after teaching and categorised using an ethogram. Data were analysed to describe the behavioural patterns before, during, and after teaching to determine whether there was stress behaviour shown and any habituation and to investigate which factors influence this stress behaviour. For maintenance behaviour, data analysis was conducted to determine if there was a difference in behaviour before and after teaching and if there was an individual difference between the sheep.
Results
A decrease in overall stress was observed across all sheep between the first and last day of use. Sheep showed individual differences between the amount of stress behaviour and the type of stress behaviour they displayed. The sheep showed increased lying behaviour after being used for teaching.
Conclusions
Sheep used for clinical examination classes were stressed, with individual differences in the type of stress response and the amount of stress behaviour expressed. The amount of stress behaviour could depend on the environment in which they are housed in before, during and after teaching. Increased lying time after teaching may indicate that sheep find being used for teaching tiring. These findings can be used to mitigate stress in animals used for teaching.

1619 - Application of New Portable Anesthesia Machine with an Oxygen Generator in Bovine Clinical Field

Author: Hyoeun NOH , Jiyeon KIM, Eunwoo CHUN, Jungha LEE, Donghwi SHIN, Woojae CHOI, Younghye RO, Danil KIM, Inhyung LEE

Objectives

Anesthesia with injectable agents has the advantages of relatively low cost and easy utilization because it does not require special anesthetic equipment. Inhalation anesthesia is a preferred method because of the ease and speed in adjusting the depth of anesthesia and the safety of anesthetic maintenance.
Currently, the only method used to sedate and anesthetize the cattle is the former one, and if sedation is needed, xylazine is sufficient, but local or general anesthesia are used together after sedation. However, if the patient is positioned in lateral recumbency after injection, there is a risk of aspiration, and recovery may be delayed due to the accumulation of drugs with repeatable injection. Accordingly, inhalation anesthesia in cattle can be considered, and a portable anesthesia machine is required to attempt inhalation anesthesia in the field. However, conventional portable devices need oxygen cylinder, which are difficult to transport, have a limited amount of gas available, and can be dangerous. As an appropriate technology, in this study, inhalation anesthesia with an oxygen generator was conducted in bovine clinical field.

Material and methods

A new portable anesthetic machine (36 kg) with an oxygen generator supplying 10 L/min (27 kg) was recently developed. Using this machine, inhalation anesthesia was performed on three calves, 48, 50, and 80 kg respectively. Each calf was sedated with intravenous xylazine (0.1 mg/kg) and positioned in sternal recumbency for intubation within 5 to 10 minutes. The sizes of endotracheal tubes were 8.5, 8.5, and 11 mm internal diameter respectively, depending on body weight.
In calf 1, vaporizer setting (isoflurane) was increased from 0 to 3% and gradually decreased to 0% for 40 minutes. The oxygen flow rate was set at 5 L/min and reduced to 3 L/min after 30 minutes, maintaining the rate before extubation. Calf 1 was healthy and did not proceed with any other treatment.
According to incorrect flexor tendon contracture treatment by owner, calf 2 needed the removal of second and third phalanges, so it required inhalation anesthesia for about 65 minutes. After intubation, vaporizer setting was raised from 0 to 3%, and maintained at 2% during surgery. At the end of the surgery, it was decreased to 1% with starting bagging and extubation was performed 10 minutes later. The oxygen flow rate started at 5 L/min and decreased to 3 L/min after 15 minutes, and this was maintained for 45 minutes during surgery. After the treatment, it was increased to 5 L/min with bagging.
In the case of calf 3, the left metatarsal bone was refractured, and it had closed pinning surgery and cast treatment. The vaporizer setting was set at 2% and maintained for about 65 minutes during surgery. The oxygen flow rate was 4 L/min after intubation, increased to 8 L/min after 10 minutes and decreased by 2 L/min every 15 minutes thereafter, maintaining at 4 L/min before extubation. Calf 2 and 3 were administered intravenous tramadol (4 mg/kg) and lidocaine (5 mL only in calf 3) infusion during surgery.

Results

In this study, anesthesia was performed by adjusting the appropriate oxygen flow rate and vaporizer setting depending on the body weight, and it took about 40, 65, and 65 minutes, respectively, from induction to recovery of the anesthesia in three calves, all of which were safely treated. In addition, it took a total of 60, 75, and 90 minutes, respectively, from the start to the end of the cases.

Conclusions

In this study, it was confirmed that a new portable anesthetic machine with an oxygen generator enables safe anesthesia maintenance and recovery even in cattle. As a result, the range of treatments that can be performed in the bovine clinical field will be broadened, and the pain of patients can be reduced more effectively during pain-related procedures. However, since this study confirmed the availability of an anesthetic device only in calves weighing 80 kg or less, further study is necessary in calves weighing more than that. It is also necessary to overcome the disadvantage of being difficult to use the machine alone.

1620 - Do farmers and vets know what a tool they have in their hands? Data reports generated by the Polish Federation of Cattle Breeders and Milk Producers in the management of dairy herds.

Author: Karolina Ferenc, Agnieszka Antczak, Maria Sady, Professor Zdzisław Gajewski, Professor Romuald Zabielski

Objectives

Polish Federation of Cattle Breeders and Milk Producers (PFHBiPM) provides the services in the field of assessing the utility value of dairy cattle since 1995. The Federation developed a health herd monitoring program based on the every month analyzing milk quality parameters, milk yield, protein, fat, urea, and since 10 years, also b-hydroxybutyrate (BHB) concentrations, together with the production data collected from the farm via dedicated software (SOL). It is useful tool to monitor health problems in the herd such as subclinical acidosis (including SARA), ketosis and mastitis as well as to control reproduction and nutrition, and improve genetic potential. In Poland, ca. 800,000 cows (39% of dairy cows) are monitored this way, but not many farmers and vets make the most of data reports in the daily herd management.

The aim of the study was to clarify how much from the data reports is applied to daily monitoring herd health by farmers and veterinarians. The second aim was to find out the obstacles in using the reports more efficiently.

Material and methods

Monthly data reports from 6 H-F dairy herds (100 to 600 milking cows) with different epizootic status, level of welfare and genetic potential from the last 3-5 years were analyzed and then discussed with farmers and veterinarians to found how the reports are of help for them in routine herd management.

Results

Data reports involving somatic cell count that indicate mammary gland problem of different intensity (acute/chronic, subclinical/clinical mastitis) in the herd as well as in individual cows were the most used. The data were helpful in preparing the decisions to take milk samples for laboratory diagnostics, start medical treatment and eventually to cull the sick cow. Changes in milk fat, protein, urea and BHB concentrations and fat to protein ratio for early diagnosis of subclinical metabolic diseases (acidoses and ketoses) in specific production groups together with indications of nutrition problems (TMR, PMR) were also given. These parts of data reports, however, were underestimated by the owners. In some herds, even 60% of cows per production group had subclinical acidosis, indicated by milk fat to protein ratio, and the owner and veterinarian were downplaying this problem. Moreover, data from milking reports showing much more information which are not so clearly visible as the animals with chronic subclinical metabolic problems, impact of the indoor climate on heat stress and milk drop or wasting of protein in TMR formulas which is not used for milk production. Moreover, data for genetic improvement of the herd not clearly mark mistakes in this area. And they are not summarized so is hard to analyze and comparison between the herds.

Conclusions

Knowledge how to read the data from the dairy herds of owners and veterinarians which are responsible for health management of dairy herds not satisfying used so additional education is needed. Moreover changes in data presentation delivered in monthly reports might be helpful to improve this analysis that is why associations, laboratories as well as by companies which supply the electronic systems for management of dairy herds should still cooperate with veterinarians and farmers to help with this analysis of data from the herd.

1621 - Impact of the on-line learning during COVID-19 lockdown on the competences of the final year veterinary students in dairy herd health management classes

Author: Professor Romuald Zabielski, Karolina Ferenc, Piotr Matyba, Maria Sady, Professor Zdzisław Gajewski

Objectives

Herd Health Management (HHM) has been taught in the 6th (final) year of studies in veterinary medicine at the Warsaw University of Life Sciences since 2017. It is the last compulsory subject after Veterinary Medicine Prevention among the so-called “clinical subjects” concerning farm animals. In the Polish university education system, all classes apart from lectures are compulsory, and absences of up to 20% of the course hours are allowed. The obligation to attend lectures is not included in the study regulations. Due to the COVID -19 lockdown the methods of teaching for almost 3 semesters changed from traditional to the on-line learning (lectures, seminars) plus a very limited number of practical classes. The aim of the study was to evaluate the practical competences of the final year students, who previously studied under the conditions forced by COVID-19 pandemic, during visiting dairy farms in Herd Health Management classes.

Material and methods

Yearly Health Herd Management is provided at Warsaw University of Life Sciences to ca. 140 veterinary medicine students by the same team of teachers. Due to the COVID-19 pandemic, teaching classes from March 2020 to June 2021 were conducted using MS Teams on-line courses. From October 2021, "normal" teaching of the HHM has returned in the form of lectures, laboratory exercises and several full-day study trips to dairy cattle barns.

Results

In the post-pandemic period, a decrease in attendance at lectures was noticed, from approximately 60% present to 5-10% present at lecture. A decrease in attendance at lectures was also observed in other subjects, but it was not that drastic. On the other hand, students took part in laboratory and practical field classes with as much commitment as before the COVID-19 pandemic. Their goal was to prepare reports on the comprehensive assessment of cow health on several large dairy farms differing in milking technology and production results (e.g., milking carousels vs. robots, TMR vs. PMR). Students independently and relatively correctly analyzed body condition scores (BCS), hoof and udder health, diarrhea in calves, TMR and herd result reports (milk production, metabolic diseases, reproduction) just like the yearlings before the pandemic. However, the report summaries and the recommendations issued to farmers were of much lower intellectual value compared to the reports prepared by the students before the COVIS-19 pandemic. The report conclusions were shallow, and the recommendations were written plain language, using in extremely general terms, like e.g. "improve nutrition" instead of proposing specific changes in the preparation and composition of TMR. According to the lecturers, the differences resulted from clear deficiencies in knowledge in the field of dairy farm infrastructure, animal husbandry, feed science and nutrition of dairy cows, practical immunology, metabolic and reproduction diseases - knowledge that students should have acquired in the earlier

years of study, and its elements should be able to use in HHM. Similarly, the pass rate for the subject in the first term dropped from 90% to 60%.

Conclusions

In conclusion, on-line teaching during lockdown resulted in general reduction of competences of veterinary students. In the following two years students did not cath-up the gap.

1804 - A NUTRITIONAL EXPLANATION FOR WEAK NEONATAL CALF SYNDROME

Author: Juan Manuel Cervantes Sanchez

Objectives

The objective of this work was to find a suitable content to motivate students to study this subject.

Material and methods

The material used was the current programmatic content of the subject of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico, and the method was to select the topic of metabolic diseases of animals and finally focused on glycogenosis, then a retrospective comparative analysis of the occurrence of neonatal mortality worldwide in different species, but with emphasis on ruminants.

Results

The results yielded valuable information. Within metabolism, glycogenogenesis and glycogenolysis play a very important role in the energy homeostasis of animals. Neonatal mortality occurs in mammals, birds and fish, and has multiple origins: infectious (Adenovirus), non-infectious (genetic, poor birth management, poor maternal nutrition, poor hygiene, etc,) In humans it is called glycogenosis, in different parts of the world in cold, temperate and tropical climates, affecting the Brahman, Shorthorn, Charolais, Simmental and Holstein cattle breeds, in which forms II glycogenosis (accumulation of glycogen in the heart), and forms V and VII (affecting skeletal muscle) have been observed, It occurs mainly in neonatal male calves (since it is a disorder associated to sex), which show great weakness, and anaerobic glycolysis is activated, which causes the calves to become paralyzed and unable to get up, besides having the jaw locked and unable to suckle and in some cases nervous signs, finally due to the lack of food they die in less than a month. In addition to hereditary defects, there are several external factors that affect the gene responsible for the production of the enzyme acid alpha-glucosidase: 14 different forms are known. In the case of animals there are reports of this disorder in intoxications by chemicals, zineb, furazolidone, prednisone, heavy metals, ochratoxins, as well as intoxications by toxic plants: Castanospermum australe, Astragalus mollisimus, Swainsonine, Atelea, glaziovana.

Conclusions

The conclusions of this work are that this disorder is more frequent than we imagine, perhaps we have not paid attention to it. It is a very good example of how genetics and metabolism interact and that there are now a number of factors that can modify genes and alter the energy metabolism of animals.

1820 - NUTRITIONAL MYOPATHY IN A SHEEP

Author: Victoria Elizabeth Castrellón Ahumada, Guadalupe Monserrat Ramos Castrellón , Gabriela Espino-Moreno , Juan Antonio Ramírez Chequer , José de Jesus Rangel de la Rosa

Objectives

Development of the clinical case based on the inspection of a corpse and histological review of the lesions observed. We sought to establish the etiology of the clinical case as well as the impact it has on the production system from which the specimen comes.

Material and methods

In this clinical case report, the death of a sheep due to nutritional myopathy is reported. Nutritional myopathy is caused by a dietary deficiency of selenium and vitamin E, it is commonly known as “white muscle” disease. Generally affecting animals younger than 6 months, this deficiency damages the myofibrils of the skeletal muscles and the myocardium, which is fatal. On March 27, 2023, the necropsy was performed on the sheep from the community of La Purísima del Rusio, Villa Hidalgo, Zacatecas. This was carried out in the Academic Unit of Veterinary Medicine and Zootechnics of the UAZ. The diagnosis was obtained after reviewing the macroscopic and microscopic lesions which coincide with the lesions of this disease, as well as the clinical signs that the sheep showed.

Results

Macroscopic findings

The necropsy was performed with the aim of finding significant lesions that could lead to a presumptive diagnosis. The most significant findings are described below:

Poor body condition was observed. The brain was observed with petechial hemorrhages in the right hemisphere, in the frontal, parietal and occipital lobes. The middle and ventral nasal conchae were found to have generalized congestion. The left costal musculature was pale. Heart with pericardial effusion and paleness at the apex of the heart

The left lung was observed with multifocal hemorrhage in the caudal lobe, the right lung had generalized congestion due to hypostasis and when the cut was made through the trachea, severe pulmonary edema could be observed. Kidneys with paleness at the upper and lower ends

Microscopic findings

Severe generalized congestion, perivascular edema, and mild multifocal hemorrhage were observed in the brain. Neuronal hyperchromasia, gliosis, perineuronal edema, neuronophagia. Nasal turbinates with hemorrhage, presence of plasma cells indicating an active viral infection. Necrosis of hair cells, severe diffuse congestion. Thickening of the pleura, hyaline content in bronchioles, congestion, severe edema, hemorrhage, proliferation of inflammatory cells and severe diffuse atelectasis. Severe dissociation and degeneration of cardiac muscle fibers, severe congestion, mild focal lymphoid infiltrate. Severe congestion in the liver, hemorrhage, mild dissociation of hepatic cords, lymphoid cell infiltrate. Kidney with hemorrhage, multifocal lymphoid infiltrate and tubular degeneration.

Conclusions

We consider that the macroscopic and microscopic findings obtained in this necropsy can be related to the fact that the sheep had a metabolic change due to the deficiency of selenium and vitamin E, which caused heart failure, resulting in pulmonary edema, leaving it exposed. to secondary pathogens, which caused inflammation and widespread systemic failure leading to death.

AMMVEB SYMPOSIUMS AND WORKSHOPS

Reproductive biotechnologies in cattle

Accelerating Sustainable Genetics Faster with Hormone Free IVF Nina Casagrande, DVM with Universidade Estadual de Londrina (UEL)

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ABSTRACT: The Global Cattle industry is facing a triple challenge. The demand for global protein, the requirement to produce in a sustainable way and one that ensures producer profitability. Genetic progress is a permanent and compounding solution to this challenge. Historically, genetic progress has been slow and inefficient in the cattle industry due to three main reasons. First, due to the lack of labor required and access to modern reproduction technology, second the limited amount of reliable consistent data and finally unpredictable results. Understanding the challenges and opportunities as to how the global cattle industry can identify elite genetics and rapidly reproduce them using modern hormone free IVF technology will be discussed. We will review the current global trends in the embryo market, explore historical experiences and dive into the latest technology developments driving change in the beef and dairy markets. Topics include:

• Reproductive Technologies Comparison

• The use and experience of Hormone free IVF – no FSH required

• The importance of standards, protocols and techniques for ovum pick up (OPU) • Latest developments in IVF culture media and the impact of outcomes

• The large-scale adoption of IVF driven by the use of Direct Thaw Embryo transfer technology to increase accessibility

• The impact of IVF to drive sustainable beef and dairy supply chains

ustainable livestock systems: towards the fulfilment of the Sustainable Development Goals (SDG)

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Abstract

Cattle production is one the main causes of land use change in the tropics. The demand for meat and milk are increasing globally and as a result, it is urgent to work on sustainable livestock production systems that satisfy food security and protect the environment. Silvopastoral systems (Ssp) are an alternative for more sustainable forms of production. Hence, their sustainability performance compared to conventional systems need to be evaluated. The SAFA framework is a tool to assess sustainability performance in agricultural systems that has not been used in livestock production. The objective of this study was to use this framework to compare silvopastoral, native (NSsp) and intensive (SSPi) and monoculture (Mc) cattle farms in the state of Yucatan, Mexico. Animal welfare was assessed using the Welfare Quality tool. Species Diversity and Richness was also obtained, as well as soil variables. Questionnaires were created using the SAFA Guidelines version 3.0 and applied in 9 farms of the local communities in 3 municipalities. Responses were fed to the application SAFA tool version 2.1.50 and sustainability polygons were produced. Native farms had positive ratings for Participation, Land, Biodiversity and Cultural Diversity, and SSPi for Holistic Management. Native farms had limited ratings for Decent Livelihood, due to poor training and underpay. NSsp farms and one Sspi farm had the highest percentages of themes positively valuated (NSsp1- 67%, Sspi- 62%, NSsp2- 57%) compared to monocultures and with one SSpi farm ranged as the lowest in positive valuations (Mc3- 33%, SSpi3- 33%, Mc2- 24%). Positive evaluations identified native systems as an option for sustainable production, however, it was also recognized that group participation, environmental knowledge and awareness, identification of potential business risks, technical support and training were key factors to improve sustainability in all farms. This study is a novel approach to the SAFA protocol, as it validates its efficiency in the conditions present in the Mexican tropics and compares the sustainability performance of Ssp systems, which have never been evaluated using this tool. Transdisciplinary research is needed to integrate measurements on sustainability criteria in different livestock production systems to design more efficient policies and incentives for producers.

Keywords: sustainability; anima welfare, cattle; SAFA; silvopastoral

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Mitigation of greenhouse gas emissions in livestock production

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Introduction

Climate change is inducing considerable economic losses in many countries due to the impact of severe droughts, heavy rains, floods and fires on animal production. Developing countries face tremendous challenges regarding adaptation and mitigation to climate change since there is an increasing demand for meat and milk by the burgeoning population. Climate change is the result of the anthropogenic emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), the main greenhouse gases (GHG) which accumulate in the atmosphere. Emissions of methane and nitrous oxide from livestock production account for a considerable proportion of total GHG emissions in the AFOLU sector of IPCC. Methane is a potent GHG which is eructated in large volumes by ruminant species and represents an energy loss of 2-12 % of gross energy intake. Methane is synthesized in the rumen through the reduction of CO_2 by the excess metabolic hydrogen (H_2) in the methanogenic archaea. Methane has a global warming potential 28 times that of carbon dioxide and its average lifetime in the atmosphere is 12 years. Several tropical legumes containing secondary metabolites such as condensed tannins and saponins have been used to assess the mitigation potential of foliages and pods (Ku-Vera *et al*., 1999) when fed in practical rations to ruminants. Results hereby presented, summarize the work carried out at the Laboratory of Climate Change and Livestock Production (LACCLIGA) of the University of Yucatan, Mexico during the last ten years.

Strategies for enteric methane mitigation in ruminants

Most of the experimental work hereby described was carried out in the open-circuit respiration chambers at LACCLIGA, University of Yucatan in Mexico (Canul-Solis *et al*., 2017; Arceo-Castillo *et al*., 2019). Prior to their adoption or implementation at farm level, a methane mitigation strategy must ideally induce: a significant extent of mitigation demonstrated in various experiments, published equations applicable to various types of ruminants, feasibility of adoption by farmers, cost-effectiveness analysis and repeatability (Eugène *et al*., 2019). Before a methane mitigation strategy is put into practice in farms, it is crucial to have precise accounts of methane inventories. The use of an erroneous approach for methane accounting may lead to incorrect calculations of enteric methane inventories and consequently to biased recommendations for appropriate mitigation practices (Ku-Vera *et al*., 2018). Considerable effort has been invested in the attempt to work out and predict accurately methane emissions under particular production systems and feeding scenarios in order to arrive to correct, unbiased; inventories of enteric methane for a given region or indeed a country. Sophisticated modeling and statistical analysis have been applied to data in the attempt to minimize uncertainty in the prediction of methane emissions (Angeles-Hernandez *et al*., 2024). As regard to mitigation of enteric methane emissions, Piñeiro-Vázquez *et al*. (2018), observed an effect of adding quebracho powder (1- 4% of DM offered) on methane yield in cattle fed a ration of low-quality tropical grass. In a subsequent experiment, Piñeiro-Vázquez *et al*. (2018) fed crossbred heifers a basal ration of tropical grass (*Pennisetum purpureum*) and increasing levels of chopped foliage of *Leucaena leucocephala* and were able to induce a decrease in methane yield when the legume was included in high proportions of ration dry matter (40-80%), this agrees with data reported by Harrison *et al*. (2015) who similarly found a reduction in methane emissions in cattle fed *Leucaena leucocephala* in Australia. In a set of experiments involving the incorporation of either foliage's such as *Leucaena leucocephala* or *Gliricidia sepium* (Molina-Botero *et al*., 2019) or ground pods of *Enterolobium cyclocarpum* or *Samanea saman*

(Lazos-Balbuena, 2015; Valencia-Salazar *et al*., 2018) it was possible to record a reduction in methane yield of different magnitudes in cattle. It is in this context, that, if it is assumed that crossbred (*Bos indicus* x *Bos taurus*) dual-purpose cows grazing silvopastoral systems consume ~34% of forage (DM) as *Leucaena leucocephala* (Bottini-Luzardo *et al*., 2016), and assuming that the reduction in methane yield could be in the order of ~20% in cattle fed *Leucaena leucocephala* (Montoya-Flores et al., 2020), then it follows that the actual mitigation of methane emissions in grazing conditions may range at approximately 15 to 20%, compared to an only tropical grass ration. Earlier work at this laboratory with the stoichiometric approach with sheep, had suggested that methane yield could be decreased by feeding ground pods of tropical plants such as *Enterolobium cyclocarpum* and *Acacia pennatula* (Briceño-Poot *et al*., 2012) at 45% of ration dry matter and obtaining weight gains in excess of 230 g/lamb/day. Those plants contain either condensed tannins or saponins, or both. The pods can be easily collected from the floor by hand by smallholders, stored in bags, ground and directly fed to cattle and sheep up to 50% of ration dry matter (Briceño-Poot *et al*., 2012). Pods are highly palatable and they are well consumed by cattle and sheep. Rumen dry matter degradability (a + b) of ground pods is high: 87% for *Enterolobium cyclocarpum* (Piñeiro-Vázquez *et al*., 2013) and 86% for *Samanea saman* (Valencia-Salazar *et al*., 2018), therefore they provide the necessary energy for the maintenance and growth of bacteria in the rumen. This high extent of rumen degradation of tropical pods suggest that they contain a high concentration of readily available carbohydrates, which may change the pattern of rumen fermentation and decrease methane production per unit of dry matter consumed. There are several possible explanations for the mitigation of methane emissions as a result of feeding foliages and pods of tropical plant species to cattle and sheep. It could be that the condensed tannins contained in such plants reduce apparent digestibility of feedstuffs (Piñeiro-Vázquez *et al.,* 2018), thus the amount of potentially fermentable carbohydrate (i.e. NDF) in the rumen is decreased. It has been reported that saponins in trees such as *Sesbania sesban* are toxic to protozoa (Newbold *et al*., 1997). It could also be that saponins in pods such as those of the legume tree *Samanea saman* may form complexes with sterols in the protozoal membrane surface and induce lysis of such membrane (Anantasook *et al*., 2014), thus reducing the population of methanogenic archaea which are usually associated to protozoa. It has also been observed that incorporation of foliages and pods in ruminant rations which contain condensed tannins and steroidal saponins have the capacity to increase the molar proportion of propionic acid in rumen liquor decreasing the availability of metabolic H_2 which is a specific substrate for methane synthesis. (Albores-Moreno *et al.,* 2017; Piñeiro-Vázquez *et al.,* 2018). Methanogens exist as exo- and endosymbionts to protozoa which allows active elimination of H_2 and therefore methane synthesis. The feeding of starch containing ingredients in the ration such as those in the pods of the legume tree *Samanea saman* (rain tree) may also drive a reduction in methane emissions by changing the pattern of rumen fermentation, increasing the proportion of propionic acid and lowering that of acetic acid in the rumen. Ground pods of *Samanea saman* (rain tree) can be used for the feeding of dairy cows and for enteric methane mitigation (Anantasook, Wanapat and Cherdthong, 2014). Changes in the molar proportions of volatile fatty acids in the rumen of animals fed plant resources (seeds, pods) containing starch may alter positively the efficiency of metabolizable energy absorbed from the rumen (Blaxter, 1962). Recently S. Valencia-Salazar (unpublished), fed rumen cannulated heifers, foliage of *Bursera simaruba* containing high levels of condensed tannins either alone (20% DM) or mixed (10% DM) with foliage of *Brosimum alicastrum* (10% DM), observing a reduction in the emission of enteric methane of ~21% for the treatment with foliage of *Bursera simaruba* alone. There are several other plant resources (foliages, seeds, pods) which may be tested for their potential to mitigate enteric methane in the tropical regions of the world. Reduction in the emissions of greenhouse gases arising from ruminant livestock may contribute to comply with commitments signed by many countries in their NDC's in the context of the Paris Agreement. Eventually mitigation of enteric methane may contribute to increase the offer of

ecosystem services by farmers and probably to participate in the carbon trade system when considerable amounts of CO_2e are produced in a given geographical region. It is unlikely that the energy saved in the rumen when methane emissions are reduced is reflected into animal product (weight gain/milk production). Nonetheless, Melgar *et al.* (2021) have speculated that a reduction in methane emissions of 93 g/day (~26%) when the potent methane mitigating additive Bovaer (3-NOP) is incorporated in the ration of dairy cows, may potentially translate into 0.39 kg milk per day in the bucket of the dairyman. However, this rationale has been recently challenged by Morgavi *et al*. (2023) in relation to the fate of the excess metabolic H_2 remaining in the rumen when methane mitigating foliages or additives are incorporated in the ration of ruminants.

Concluding remarks

In summary, when foliages and pods of tropical plants were incorporated in the ration of cattle and sheep, methane yield (g CH_4/kg DMI) was decreased from 15 to 25%, depending on plant species and percent incorporation in ration dry matter. *Leucaena leucocephala*, a legume shrub native from Mexico, seems to rank among the best options to mitigate enteric methane in tropical regions. Based on the results of the experiments performed, it can be concluded that condensed tannins and saponins, as well as starch contained in foliages and pods of tropical trees and shrubs represent a viable option to mitigate enteric methane emissions under practical farming conditions. Strategies of enteric methane mitigation in ruminants fed low-quality forages can effectively decrease enteric methane emissions per unit product (weight gain/milk yield), reducing the contribution of livestock to greenhouse gas emissions and therefore to climate change. It seems unlikely that the energy saved by methane mitigation will transpose directly into animal productivity and more work is required to better understand the thermodynamic relationships among: heat of fermentation, microbial growth and volatile fatty acids stoichiometry in the rumen. The increasing demand for animal protein arising mostly from the developing world, can be realized rationally, if the emissions of greenhouse gases such as methane and nitrous oxide are reduced. Plant resources available in many regions of the world present ample possibilities for diminishing emissions of GHG from ruminants which contribute to climate change.

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Cattle Health, Husbandry and Genetics: the Sustainability Connection

Ruaraidh Petre

Abstract

The global food system faces increasing challenges, needing to reduce the impact of the current system while feeding a growing population. Livestock production is part of this, and animal health, breeding and genetics can contribute significantly to livestock sustainability. The Food and Agriculture Organization of the United Nations (FAO)'s (2023) report gives guidance on possible improvements in the livestock supply chain, while increasing food accessibility. Key takeaways include productivity increases (per animal) and breeding (reducing calving interval and using better genetics) which are the major contributors to reducing the overall footprint. Adoption of cattle vaccinations against common diseases can reduce the footprint and the size of the supporting herd. We urgently need investment in all aspects of animal health and welfare to help drive the combined benefits to human lives, health and livelihoods that better animal production can deliver.

Introduction

Animal Health and Animal Welfare are part of the definition of Sustainable beef. Without caring for cattle properly, we cannot claim that we are operating sustainably. This is an ethical imperative and one that bears emphasising from the beginning. We will look at the ways in which health and welfare reinforce each other, and contribute to a number of aspects of sustainability. We will also look at how actions that may be primarily targeted at sustainability can reinforce health and welfare.

First, however, remember it is the ethical imperative that there is no sustainability without animal health and welfare. We have a duty of care to the animals we raise for food, and everyone involved in the process needs to be doing what they can to give them a life worth living.

Sustainability

The global food system faces increasing challenges, needing to reduce the impact of the current system while feeding a growing population. At the same time, food production itself is affected by climate change. Uniquely amongst industries, food production has the ability to contribute to both adaptation and mitigation of climate change. Livestock are very much par

As time goes on, we will find increasingly that areas that were capable of producing crops becoming unable to do so, due to climate change. As it is, the majority of land we have available for agriculture cannot grow human edible crops. Ruminants can upcycle roughage that we can't eat into highly nutrient dense meat.

All industries need to be working on reducing emissions, and the livestock industry has been on a decreasing emissions trajectory per pound of beef for decades, continuing into the present and beyond. These efforts are in both High-Income Countries, and in countries such as Brazil. There are numerous ways we can reduce emissions associated with beef production, in intensity, and if adopted widely enough, in absolute terms. Despite widespread public commentary, the details of how beef supply chains can reduce emissions are still not well understood by those outside the industry.

The Global Roundtable for Sustainable Beef (GRSB) and our members have set an ambitious goal for GHG emissions: A reduction of 30% by 2030, on a pathway to net zero. The short-lived nature of methane as a GHG means that emissions do not have to cease entirely to stop

warming. In fact any reduction in emissions from a given herd means that the herd is contributing to reduced temperature forcing.

It will not be easy to accomplish this on a global scale, primarily because increases in demand in developing countries stimulate increased supply through larger herds. We need to accelerate investment in those regions of the globe to ensure that the increasing demand from larger human populations is met in sustainable ways, by producing more efficiently rather than expanding the herd. FAO's report (FAO 2023) released during COP28 outlines how increased global demand for meat can be met without increasing emissions but decreasing them. GRSB set our goal to focus minds and investment to rise to this challenge, and we are bringing participants in the supply chain together to identify and address common barriers. Goals to reduce GHG emissions have also been set in many parts of the food system. Providing transparency and insights on good husbandry, health and welfare along the chain also contributes to greater value for both primary producers and buyers (Maia de Souza *et al*. 2017).

This brings us to how Sustainability is defined. The answer to this question is exactly what a group of farmers and ranchers, processors, input providers, retailers and NGOs wanted when we formed the Global Roundtable in 2012.

It took GRSB 2 years to finalise our definition, which was agreed upon by our members and launched at the Global Conference on Sustainable Beef in Sao Paulo in 2014. It encompasses 5 principles covering Natural Resources, Animal Health and Welfare, People and the Community, Food, and Efficiency and Innovation. It also includes 36 criteria, and the document is 11 pages long. That might seem a bit unwieldy, but it's important to understand your priorities before embarking on a journey, particularly one as large as transforming a significant industry feeding billions of people.

GRSB defines sustainable beef as a socially responsible, environmentally sound, and economically viable product that prioritizes Planet, People, Animals and Progress.

When asked to come up with a brief definition of my own for "sustainable production" I use "Production that meets the needs of today's population without compromising our ability to meet future needs," which aligns with GRSBs and with terms such as regenerative or agroecological.

In 2021, GRSB launched three global goals covering Climate, Nature Positive Production and Animal Health and Welfare. This year we will launch our 4th goal on Social Impact.

These days, it is hard to open a newspaper or a newsfeed without reading an article about how modern agriculture impacts the planet, mostly in the negative. First among the culprits identified is usually beef production, and the main complaint is regarding beef's enteric methane emissions, followed by deforestation / land conversion. We have to acknowledge those issues, and like every other industry, we do have to work to minimise the negative impacts of our production on nature and climate. Having said that, we also need to acknowledge that people need to eat and that 2/3 of our agricultural land cannot produce human edible crops.

Ruminants upcycle grass and other roughage into nutrient dense meat with highly bioavailable minerals and vitamins that are deficient in significant portions of the human population. The current narrative is damaging because it offers no solutions, while pitting the consumer against the farmer. Many of the problems we see in modern agriculture actually do not stem from farmers, but from policy and other signals they receive from governments and companies further up the supply chain. The consumer pays less for food now than they did in the 1970s (in constant pricing), and it is generally the farmer that has lost most of that income.

So, while avoiding "Carbon Tunnel Vision", what does animal health and welfare contribute to reducing the impact of beef?

This is not purely a question of efficiency, but efficiency is a very significant contributor to it. Consider the dairy cow that can supply milk and a calf that can be finished for beef every year. Contrast that cow with one that has insufficient feed and health care, to the extent that she only produces a calf every 4 years. The first dairy cow has a much lower footprint per kilo of product than the second. In emissions per year, the first dairy cow is probably higher, but the other cow has produced nothing.

Now consider an expanding herd: Without adequate inputs, feed, healthcare and good husbandry we see a huge impact, due to the short-lived nature of methane. Contrast that with a herd that has increased production per head over three decades while *decreasing* in size. The latter has actually contributed to lowering climate impact, while feeding more people.

Resilience

The concept of resilience is defined as "a system property that, in its most desirable form, allows thriving systems to cope with change. The systems perspective blends ideas from complexity theory, ecology, and social sciences to understand dynamics of change and alternate regimes in social-ecological systems." (Hodbod et al 2022). As it happens, measures designed to increase resilience and adapt to climate change may also favourably benefit biodiversity in agro-ecosystems, as well as assisting in mitigating climate change: "They're not really about carbon farming, even though that's an outcome... They have a focus on rebuilding resilience into the landscape and with that comes productivity." (Gosnell et al. 2020).

The framing of sustainability in terms of resilience gained considerable ground as a result of the Covid-19 pandemic which coincided with the widespread epidemics of African Swine Fever (ASF). What both diseases demonstrated was that shocks at this scale have a very disruptive effect on supply chains with negative impacts on consumers and producers. While value chains can be efficient when there are no disruptions, 2020 showed they are poorly adapted to cope with such large human or animal disease outbreaks (Hodbod *et al* 2022), underlining the keystone importance of animal health and zoonoses in sustainability.

Animal Health and One Health

Following on from the concept of resilience as outlined above, the interrelation between animal and human health is critical. The example of twin shocks to the food system of Covid-19 and ASF underlines how both zoonoses and livestock disease can impact human lives and livelihoods.

I have had the good fortune to work with livestock and people in a wide range of different environments. In each of those contexts, animal health was a major focus for me and the people I worked with.

In my early career I worked on and managed sheep and beef farms, where vaccinations, anthelmintics and acaracides all played their part in ensuring those farms were productive and profitable. Given the very low returns in farming in general, and livestock in particular, these routine treatments made the difference between a viable farming system and one that could not pay for itself.

In Scotland, we vaccinated against a range of clostridial diseases and BVD, and regularly rotated our anthelmintic treatments to try to avoid the development of resistance. At that time, there were cases of BSE in the country, so we had to be particularly careful where we sourced our cattle. As we were in the west of the country, in a very wet climate, we also had persistent issues with foot rot in sheep that required a high degree of vigilance and development of a pasture rotation to help minimise the problem. Mineral supplementation

was important in cattle and sheep, with both copper and magnesium required on those pastures.

In Australia, I worked on some very extensive properties in the Northern Territory. On one, we had to vaccinate against botulism, and those were still the days of regular Brucellosis and Tuberculosis testing. One of the routine jobs, next to mustering, branding and drafting, was the TB intradermal caudal fold test, with all of the reactors destroyed. That government program ultimately led to the eradication of TB from Australian herds (Glanville, 2023).

I also worked on a program that included rinderpest vaccination in Southern Sudan as part of the global rinderpest eradication effort (Catley, Leyland & Bishop 2005). Following that. I ran a veterinary program in Afghanistan (Schreuder, 2020) which included paravet training, and the import and distribution of veterinary vaccines and medicines covering the whole country. As Afghanistan still has endemic anthrax, and both Peste des Petits Ruminants (PPR) and Crimean Congo Haemorrhagic Fever (CCHF) were present in the country, the importance of having a good network of paravets and a reporting system under relatively primitive conditions cannot be overestimated.

In each of these contexts there was never any doubt about the importance of animal health to the livelihoods of the people involved, and the sustainability of the system. The paravet model in Afghanistan emphasised the importance to those communities. Their level of understanding of herd immunity was demonstrated by villages paying for vaccination of all animals, ensuring that all were covered and paid for, even when individual livestock keepers could not afford vaccine.

Yet there are large areas of the world to this day where availability of vaccines and treatments is limited, where the distance livestock keepers travel makes it unrealistic that they will keep up regular vaccinations or treatments required. Simple problems like copper deficiency in sheep resulting in swayback in lambs remain unrecognised or untreated simply due to a lack of available qualified people on hand to recommend supplementation. In Africa and Asia, with over two thirds of the large ruminants in the world, they produce less than one third of the products from them. We urgently need more investment in veterinary medicine in lower income countries to improve the sustainability of animal production and the availability of nutritious animal source foods for the growing population.

If you have not read the Oxford Analytica's Health for Animals report that came out last year (Oxford Analytica, 2023) I thoroughly recommend it. I will give you some examples from it that elaborate on the impact of health on sustainability:

> "A 60% global vaccination rate for beef cattle correlates to a productivity rise of 52.5%. This rise is equivalent to the beef consumption needs of 3.1 billion people."
>
> "Globally, the modelling found that a 40% global vaccination rate for cattle in a given year is associated with a 5.2% reduction in land required for production."
>
> "Every 1% reduction in beef cattle disease rates would: a) increase production enough to meet the average beef consumption needs of 317 million people b) increase producer revenue by US$3.2 billion."

I used the heading One Health and we should not underestimate the interaction between animal health and human health. It is not just through zoonoses but also through the impact of good stewardship of pharmaceuticals that ensures they remain effective for both people and animals. Antimicrobials are the products where most concern is expressed about use in animals, and livestock keepers and their vets need to make certain they minimize the use of critically important antibiotics for human health. Needless to say, doctors treating people should be similarly cautious in their use of critically important antimicrobials.

In beef cattle, the products of most concern are macrolides due to their importance in human medicine and their use in Bovine Respiratory Disease. This emphasises the need to condition cattle, vaccinate them and get them as resistant to BRD as they can be to minimise the need to use macrolides both in pastured cattle and in feedyards. (BRD is the most common reason for antibiotic treatment before weaning).

Animal Welfare and Husbandry

Everyone instinctively understands how good animal health contributes to animal welfare. We have all been sick at some stage and recognise that when sick, we are not feeling good. It fair to assume the same situation exists for animals. Not surprisingly, this is confirmed through animal behaviour, appetence, cortisol levels etc. No surprises there. What about the other way around? Does improved welfare contribute to better animal health?

To a room of veterinarians, the answer should not surprise you: Better welfare certainly leads to better health outcomes. There are some concrete examples of how that works in practice, Painful procedures like disbudding, when done early with anaesthesia and analgesia, lead to calves that eat better and are less likely to succumb to infections, as compared to those without. Similarly, fenceline weaning is recognised as being less stressful on calves leading to lower levels of movement and increased appetence. This contributes to producing calves better conditioned and resistant to disease prior to later transport for backgrounding or finishing. Apart from the ethical reasons for good welfare stemming from good husbandry, it makes good financial sense for all those in the chain to ensure that cattle are well treated from birth onward.

Other issues may be considered good husbandry rather than welfare *per se,* such as ensuring micronutrients are sufficient through mineral supplementation, avoiding excessive poaching in fields, excessive manure and overcrowding in housed or penned cattle, all clearly contribute to welfare and directly to health. It has been demonstrated that reduced space per animal in outdoor feedlots impoverishes animal welfare (Macitelli *et al.,* 2020;

Vaccinations cannot overcome poor husbandry or poor welfare. Treating stock that are sick as a result of poor husbandry or welfare is a double and avoidable expense. Good health, feed and husbandry all contribute to reducing calving interval, a key efficiency parameter. This, in turn can reduce the size of the supporting herd and pressure on natural resources.

Genetics

Breeding and genetics can provide cattle suited to a wide range of environments. Breeds with adaptations to different climates, through e.g. *bos indicus* genes or short haired coat, or disease resistance to tryponsomosis, through African taurus cattle such as the N'dama, already exist. Clearly adaptations to where they live in terms of climate, available forage or topography and endemic diseases are the foundation of having a productive herd.

Cross breeding resulting in hybrid vigour has been used for generations to increase the output of cattle, though such cattle still have to cope with their environment. The benefit of cross breeding can be tempered by the level of adaptation of the two breeds concerned and the inputs livestock keepers are able to provide them.

More recently we have seen the introduction of genes into cattle through CRISPR gene editing. This can deliver a whole new trait without the need for cross breeding of two otherwise rather different breeds, and can thus accelerate progress in one direction. In 2022, the FDA in the USA declared cattle that had the slick coat gene edited to be safe to market. This holds a lot of promise for adaptations required to cope with a changing climate, as well as disease resistance genes that could be transferred to animals with other desirable traits including productivity, polled genes and others.

Within breeds, and indeed within individual herds, there is considerable variation in genetics that are related to sustainability. Residual Feed Intake is one of these, and biomarkers associated with RFI are now included in some EBVs and EPDs. Since feed represents around 70% of costs, this can represent a significant savings to producers in terms of feed required to achieve a given output.

Similarly there are now bulls with a low methane trait, which could reduce the methane emissions in the (initially dairy, later beef) herd by as much as 1.5% per year, with widespread adoption. The variability in methane emissions within and between herds of the same breed is considerable (CV ranging from 22% to 67% within farms, from a sample of commercial dairy herds (Bell, M.J. *et al* 2014)), there is considerable scope for selecting for low enteric methane, where the technology is available to measure this.

Individual producers make selections based on their system and priorities. Physical environment is not the only purpose of breed selection. Clearly, those using cattle for multiple purposes will have a different set of selection criteria from those who are seeking to maximise marbling or average daily gain. Preservation of genetic diversity in cattle is important as it may help us meet challenges in the future. Genomic tools that enable us to identify sustainability and production related traits can also be used to monitor and conserve genetic diversity (Eusebi *et al,* 2020).

Fortunately, the rise in availability of data on EBVs and EPDs can help producers target their selection to work in their environment and their system. Availability and adoption of AI services and genomic data is still patchy, and thus the potential for genetic advancement is far from being met in the beef industry, compared with dairy or the swine industry.

The use of beef genetics on dairy cows is making a substantial difference to the efficiency of beef production in the US now (Poock *et al*, 2021). 66% of cattle slaughtered in Europe come from the dairy herd, and the figure for New Zealand is around 40%. There is opportunity for significant savings in terms of the supporting herd through use of beef on dairy.

The genetics of the microbiome should also be considered when looking at efficiency traits. Microbiome–host interactions play an important role in efficient nutrient digestion and cattle health. Microbial markers and gene expression in the rumen microbiome are now being used to identify efficient cattle (Terry *et al* 2021).

Contributions towards lowering the hoofprint of livestock

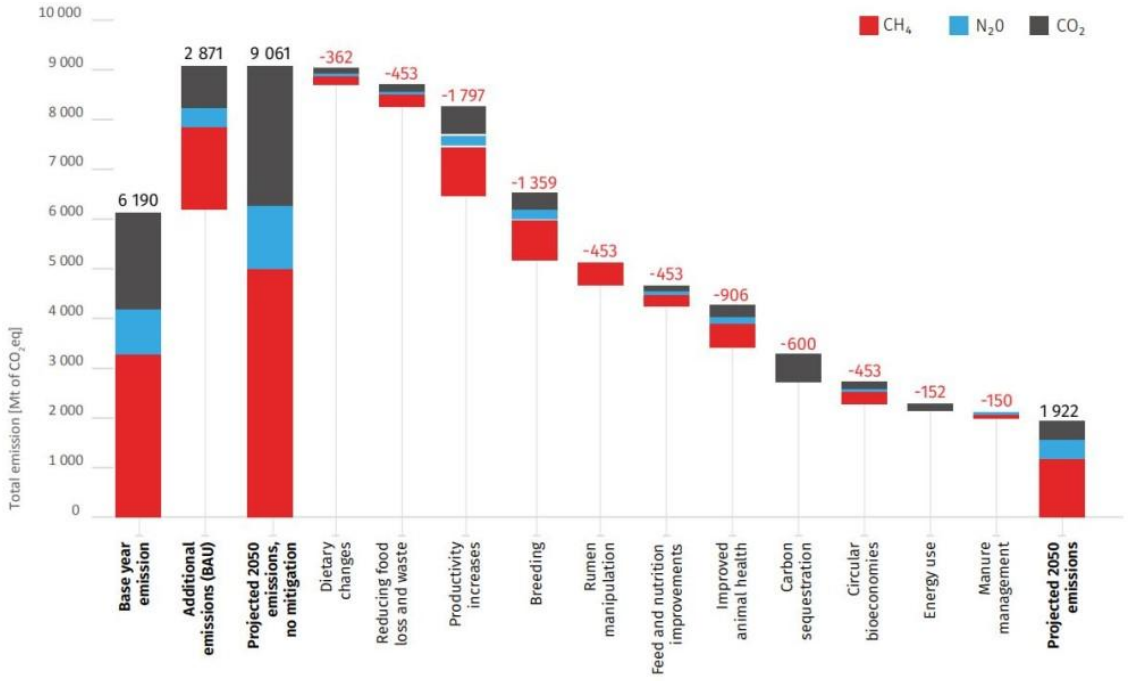

Fig 1. Base year and projected emissions from livestock systems shown as a waterfall chart with a range of mitigation measures applied to 2050 with their technical potential (FAO, 2023)

FAO released a constructive report (FAO, 2023) regarding livestock and climate impact at COP28 In it, they reported we need more meat and animal sourced food, and in order to meet demand without increasing the climate burden, there are a number of things we can do. Animal health and welfare are very much part of that picture.

The FAO report gives us some confidence that emissions reductions are possible in the beef supply chain, while increasing food accessibility. Key takeaways from that report (see fig 1) are that productivity increases (per animal), and breeding (reducing calving interval and using better genetics), are the major contributors to reducing the overall footprint. Animal health, feed quality and reducing food waste are crucial components, and on the other side of the equation, increasing soil carbon sequestration can also play a part. On top of those more classic approaches, there is some promise in terms of rumen manipulation, feed additives or vaccines to reduce production of enteric methane at its source. In some intensive systems, manure management and biogas capture can turn emissions into an energy source.

There is science to back up these FAO and Health for Animals figures. A sustainable future for *all* means investing to ensure that developing countries progress on a more sustainable path than High Income Countries have over the last 2 centuries.

It bears repeating: Animals experiencing good health and welfare are more productive than those that don't. It translates into more product per animal or per day of life, and therefore lower impact. In terms of climate impact, clearly that means lower emissions per kg. This is how productivity increases represent the largest contributor to FAOs pathway to lower emissions.

Beyond that, better health and welfare has resulted in a *smaller supporting herd* for the amount of beef, milk and leather produced. We have seen this development in many countries over the past several decades, which in turn means a smaller footprint and at least the *opportunity* for more nature positive production.

US total cattle inventory peaked around 1975 at 132 million head, and is today 94 million, a reduction of 29%, but *beef* production is 17% higher at around 12.2 million tonnes in 2022. If the US still produced in the same way as in 1975, the country would need a herd of 60 million more head than it actually has, using more water, land and feed. 60 million head of cattle represents a similar number to the total number of wild bison in North America prior to the arrival of Europeans.

All countries can make that transition and many, for example, in Latin America are following that trajectory. Brazil has intensified over the past decades, but has continued herd expansion, meaning that there are a larger number of cattle on a similar area. The total herd size in Brazil is currently around 220 million head producing around 10 million tonnes of beef in 2022. There is still considerable room for land sparing by improving productivity per animal. The connection between the expansion of the Brazilian cattle herd and deforestation is often cited, and it is certainly true that cattle are invariably present at the deforestation frontier. That is not to say that all deforestation can be attributed to beef production.

There are many factors that contribute to deforestation including timber extraction, land value speculation (the value of newly cleared land is immediately 3-5 times more than land with forest, and once it is ready for crop cultivation, that land can be worth as much as 20 times as much as forested land), and the desire to bring more land into crop production. Crops cannot be cultivated in newly felled land, so cattle are used as an intermediate stage to establish occupancy and some return until infrastructure and land condition permit cropping. This complex of drivers includes cattle, but ultimately is about economic development. In order to cease deforestation in such countries, the world as a whole needs to value forested land higher than it currently does. The total footprint of the cattle herd in Brazil has not grown since the 1990s, but it has moved with the frontier. With improved productivity, the footprint of the herd could shrink, as has been the case in the US.

While deforestation receives a large amount of attention, particularly in the popular media, grassland ecosystems are the most threatened on the planet. In many cases they are capable of producing human edible crops and are vulnerable to conversion.

Regenerative approaches to land management are an important part of reducing current and future impacts of livestock production and can contribute to ecosystem services for the water cycle, biodiversity and soil carbon sequestration. Such management has potential to reverse land degradation (Angerer *et al* 2023, Sandhage-Hofman, 2023). There are more than 1 billion ha of degraded rangeland around the world, and further large areas of degraded croplands, we need investments to encourage these management approaches. Restoration could also relieve pressure on conversion of forests and other ecosystems.

Well managed grazing can preserve and enhance the biodiversity and ecosystem functions of native grasslands, regardless of the species involved, just as a lack of management can result in degradation even where wild species are involved (Beschta *et al,* 2020). The fact that regenerative systems contribute to outcomes that help adaptation to and mitigation of climate change as well as enhancing biodiversity, leads to the conclusion that they can also contribute to economic and social dimensions of sustainability, even if they are not necessarily maximizing yield per unit area (Rowntree et al. 2020).

Within such approaches, silvopastoral systems (SPSs) (Mauricio R.M *et al.,* 2019) offer a triple win in (mainly) tropical environments. While SPSs can enhance biodiversity, carbon capture and water cycle, they can simultaneously increase carrying capacity, income and resilience of the production system. They contribute to improved animal health and welfare through reducing surface temperature and increasing shade, as well as providing a wider diversity and higher quality of feed and micronutrients. It seems clear that this is an area in which considerable investment should be directed. The establishment costs of SPSs is generally

higher than pure grazing systems, but the return on investment is only a few years and the benefits to people, planet and animals are clear.

Conclusion

To conclude, it is clear that transitioning livestock production to more sustainable models is possible, but not automatic. Current system dynamics lack resilience in the face of multiple challenges including biodiversity and climate crises. Trade arrangements that favour a race to the bottom and short term political decision making are all obstructions to transformation. We need investment in all aspects of animal health and welfare to help drive the combined benefits to human lives, health and livelihoods that better animal production can deliver.

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Brucelosis control and challenges

Control of brucellosis control using the RB51 vaccine in adult beef cows

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Introduction

Brucellosis is a highly contagious disease caused by the bacteria *Brucella sp.* (Godfroid et al. 2011). It is a zoonosis of global importance with nearly worldwide distribution, as few countries have managed to eradicate the disease. There are reports of high seroprevalence of *Brucella abortus* particularly in developing or underdeveloped countries (Megersa et al. 2011, 2012; Aznar et al. 2014; Fero 2020; Njeru et al. 2021; Rodrigues et al. 2022; Sibhat et al. 2022) In cattle it causes significant losses, mainly by causing an increase in abortions and reducing the reproductive performance of herds (Rubach et al. 2013). In humans, brucellosis is rarely fatal, but can cause substantial morbidity that requires long-term treatment (Rubach et al. 2013).

Due to the lack of vaccines for humans, prevention of infection relies on controlling the disease in farm animals. In livestock, infection occurs mainly due to the ingestion of water or food contaminated with uterine discharges, placental debris, and aborted fetuses from animals with the disease, which may spread the bacteria for up to 30 days. Thus, prevention of the disease in pregnant cattle must be a goal to control the transmission of the pathogen (Barbosa et al. 2017).

The vaccination of young female calves and prepubertal heifers (3 to 8 months old) with the S19 strain is the standard procedure adopted - for brucellosis control and in cattle of Brazil. However, this strategy is not recommended for pubertal heifers and cows, as well as for males, because it induces the production of specific antibodies that interfere in serological diagnosis and may causes abortions in pregnant cattle (Godfroid et al. 2011). On the other hand, rough strain vaccines, such as RB51, do not induce production of antibodies against lipopolysaccharide O (O-PS), and thus do not interfere with conventional serologic diagnostic methods used to test adult cattle (Schurig et al. 2002).

The RB51 vaccine has been successfully used in programs to control brucellosis outbreaks by mass vaccination of herds in countries such as Brazil (Barbosa et al.,2017), Azores (Martins et al. 2009) and Spain (Sanz et al. 2010). Therefore, routine vaccination of adult cows with the RB51 strain could be an alternative to minimize the spread of the disease in commercial herds in endemic areas. However, whether a single vaccination is effective remains to be evaluated. Thus, the aim of this study was to compare the effectiveness of brucellosis control using one or two doses of RB51 (given in 6-months interval) in commercial beef farms previously vaccinated with strain 19.

Materials and Methods

All procedures - followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999) and approved by the ethics committee. This experiment was carried out in two commercial beef farms in Minas Gerais State, Brazil, from September 2020 to June 2022. These farms were selected because of their records of endemic brucellosis and a prevalence of 6 to 8% of positive cows. Both farms vaccinated only female calves between 3 to 8 months old with the S19 vaccine. During the experimental period, cows were raised in *Urochloa sp*. pasture with ad libitum access to a

commercial mineral mixture. Farm 1 used fixed-time artificial insemination (FTAI), with inseminations and births occurring throughout the year. Farm 2 adopted a breeding season (December to March), with a first FTAI followed by natural mating using sero-negative bulls.

An initial serology (day 0) was carried out using buffered plate agglutination test (BPAT) or Rose Bengal plate agglutination test (RB) of all cows, and those with a presumptive positive result were re-evaluated using a slow agglutination test with 2-mercaptoethanol (2-ME). Specific tests to detect B. abortus. Cows confirmed as seropositive by 2-ME were culled. Non-reactive cow, in each farm, were randomly assigned into three treatment groups balanced for parity, and BCS: G1 (N=277), a single vaccination with RB51 (Bovilis RB51 – MSD Animal Health); G2 (N=283), two doses of RB51 given 6 months apart; and G3 (N=279, control group), no treatment. The cows of all three treatments groups were managed together, i.e., had equivalent exposure to environmental contamination by *B. abortus*.

Serological surveillance was carried out with RB tests performed on days 90, 180, 270 and 360. All suspected positive outcomes in the RB were checked by 2-ME, and cows confirmed as seropositive were culled. On both Farms we recorded data of abortions after vaccinations.

Diagnostic performance parameters of the RB plate test were calculated retrospectively, as reported elsewhere (Siqueira et al. 2013), using the results of the 2-ME as the gold standard. Briefly, the results of both tests were inserted in a 2x2 contingency table, considering the true positive, true negative, false positive and false negative. As the 2-ME was used only to check for false positives, false negatives were assumed to be 0, and results not analyzed.

Due to the differences in reproductive management between Farms 1 and 2 (adoption or not of breeding season), different endpoints were considered to evaluate the effect of vaccination on reproductive efficiency for each farm. On Farm 1, FTAI was carried out throughout the year, we considered the interval between calving to first AI, the number of AI per conception, and number of days open. On Farm 2, which adopted a breeding season with FTAI followed by natural mating, we could only calculate the final pregnancy rate.

The experiment was analyzed as a block randomized design. The binomial variables (occurrence of new infections, abortion) were analyzed by logistic regression procedure of SAS (SAS Institute Inc., Cary, NC), including in the model the effects of treatment, pregnancy days, parity, BCS, and their interactions. The variables interval between calving to first AI, AI per conception, and days open were compared among groups by ANOVA, using the PROC GLM, and means contrasted using the Tukey's Ad-Hoc test. Pregnancy rates were compared by the Chi-squared test. Results are shown as mean±SD or percentages. A P-value <0.05 was considered to determine statistical significance.

Results and discussion

In the current study we evaluated two strategies to control endemic brucellosis in beef cattle by immunizing adult pregnant cows with the rough RB51 strain vaccine. We hypothesized that vaccination would control the occurrence of new cases within the herd, without affecting the results of routine serological test Rose Bengal (RB). Additionally, we hypothesized that a booster vaccination 180 days later would improve protection against infection. Our results supported our first hypothesis, as demonstrated by the lack of new seropositive cows within the vaccinated groups from 180 days onwards. However, there was no evidence to support the necessity of a boost vaccine (second RB51 boost) to ensure short-term protection against brucellosis.

There was no difference (P>0.05) among treatments on the average age (in years) of cows allocated into groups G1, G2 or G3 (5.4±3.7, 5.8±3.5 and 5.6±3.3 for Farm 1 and 6.7±3.5, 6.8±3.3 and 6.7±3.7 for Farm 2, respectively). Results of the initial serological test for brucellosis using RB and 2-ME are shown in Table 1. No difference (P>0.05) was found in the prevalence of the disease between Farms 1 and 2.

Table 1: Outcomes of the first serological screening for brucellosis using Rose Bengal (RB) or 2-mercaptoethanol (2-ME) tests on the beef farms evaluated.

Farm	n	Presumptive seropositive (RB+)	Confirmed seropositive (2-ME+)
1	477	53 (11.1%)	26 (5.5%)
2	673	69 (10.3%)	38 (5.7%)
Combined	**1150**	**122 (10.6%)**	**64 (5.6%)**

The results of serological surveillance performed at days 90, 180, 270 and 360 are shown in Table 2. Seropositive cows were still detected in both vaccinated and control groups at 90 days (1/513 and 1/312 for G1 and G3, respectively). However, no new seropositive cows were detected in G1 at 180 days or in G1 and G2 at 270 and 360 days, whereas new seropositive cows were diagnosed in all exams in G3 (control). Therefore, the cumulative number of new infections was lower in vaccinated than in control cows (0.2%[a], 0.0%[a] and 3.2%[b] for groups G1, G2 and G3 respectively; P=0.0002), as shown in Figure 1.

Table 2. Results of the serological testing with RB and 2-ME of beef cows from two farms carried out 90, 180, 270 and 360 days after vaccination against brucellosis with RB51 once (G1), twice (G2) or in the control group (G3).

Day[1]	Farm	n[2]	Test[3]	G1 (1x vaccine)	G2 (2x vaccine)[4]	G3 (control)
90	1	404	RB	15/266	--	6/138
			2-ME	0/266	--	0/138
	2	521	RB	18/347	--	10/174
			2-ME	1/347	--	1/174
	Combined	**925**	**RB**	**5.4%[a] (33/613)**	**--**	**5.1%[a] (16/312)**
			2-ME	**0.2%[a] (1/613)**	**--**	**0.3%[a] (1/312)**
180	1	403	RB	13/265	--	4/138
			2-ME	0/265	--	1/138
	2	438	RB	13/295	--	5/143
			2-ME	0/295	--	1/143
	Combined	**841**	**RB**	**4.6%[a] (26/560)**	**--**	**3.2%[a] (9/281)**
			2-ME	**0.0%[a] (0/560)**	**--**	**0.7%[a] (2/281)**
270	1	402	RB	8/130	6/135	5/137
			2-ME	0/130	0/135	1/137
	2	438	RB	6/147	5/148	4/142
			2-ME	0/147	0/148	2/142
	Combined	**840**	**RB**	**5.1%[a] (14/277)**	**3.9%[a] (11/283)**	**3.2%[a] (9/279)**
			2-ME	**0.0%[a] (0/277)**	**0.0%[a] (0/283)**	**1.1%[a] (3/279)**
360	1	398	RB	7/128	6/135	5/135
			2-ME	0/128	0/135	2/135
	2	430	RB	6/146	5/144	4/139
			2-ME	0/146	0/144	2/139
	Combined	**828**	**RB**	**4.7%[a] (13/274)**	**3.9%[a] (11/279)**	**3.3%[a] (9/274)**
			2-ME	**0.0%[a] (0/274)**	**0.0%[a] (0/279)**	**1.5%[b] (4/274)**
Accumulated[5]			**2-ME**	**0.2%[a] (1/613)**	**0.0%[a] (0/283)**	**3.2%[b] (10/312)**

[1] Days after the first vaccination
[2] Numbers decrease due to culling of positive cows or for other unrelated reasons
[3] RB: Rose Bengal Test; 2-ME: 2-mercaptoethanol test

[4] Data from G2 was only considered after the 2nd vaccination, given at day 180
[5] Percentage of confirmed seropositive / original population.
[a,b] Percentages followed by different superscripts, on the same row, differ (Chi-squared or Fisher tests, P<0.05).

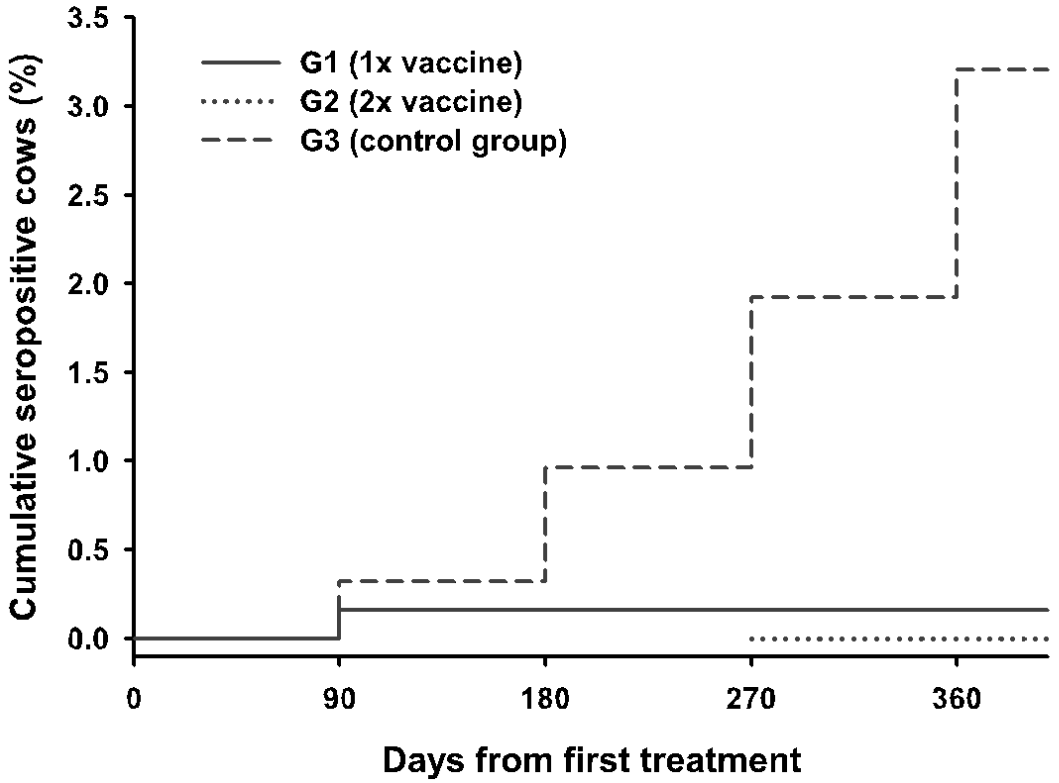

Figure 1: Cumulative percentage of cows infected after the beginning of the study.

Considering all tests carried out during the initial screening and subsequent surveillance, the RB plate? test resulted in 4.3% of false positives (198/4,582), that resulted in a specificity of 95.6%, an overall accuracy of 95.7%, but a positive predictive value of only 27.5%, when compared with 2-ME. The Kappa agreement value between the two techniques was 0.41.

The occurrence of abortion after vaccination is shown in Table 3. We observed no difference (P>0.05) in the incidence of abortion among groups.

The first concern regarding vaccination against brucellosis in adult cattle is the potential interference with routine serological tests with RB, as observed with the S19 strain, or even induction of abortion in pregnant cows. In this regard, we observed no changes in the percentage of positive reactions in the RB test following the first or second vaccinations with RB51, which indicate that this strategy would not interfere with brucellosis surveillance. Although the RB was characterized by a low positive predictive value, the overall accuracy of the test (95.7%) supports its use as a screening method, corroborating previous studies (Aznar et al. 2014; Rodrigues et al. 2022). In this regard, if vaccination with Strain RB51 would increase seroconversion, which was not observed in the current study. If it occurred then it would result in a greater demand for the use of more expensive confirmation tests, such as 2-ME. On the other hand, our results highlight that the seroprevalence reported by studies that used RB as a sole diagnostic test may have overestimated, and that led to the unnecessary culling of many false-positive cows.

Our second hypothesis, i.e., the potential advantage of a booster dose 180 days after the first vaccination with RB51, was not supported by the results. Further studies are required

to evaluate whether a second dose of vaccine would be beneficial in herds with higher prevalence of the disease, or to guarantee long-term protection in places where the disease cannot be completely eradicated, e.g., if present in wildlife.

In our post-treatment surveillance, one seropositive cow was detected 90 days after vaccination. However, this is most likely due to the testing of a recently infected individual, as no other seropositive cows were found in the vaccinated groups up to 360 days after the initial immunization, whereas new infections were observed in all subsequent tests in the control (non-vaccinated) group. As cows from all groups were raised together, this difference indicates that the pathogen was present in the environment, and vaccination with RB51 protected cows from being infected and develop the disease. On the other hand, it highlights the limitations of culling as a sole strategy to control brucellosis. In the control group (G3), new infections were observed in all exams, despite all positive cows being culled after each exam, also indicates less effectiveness of just calf hood vaccination with ST19 vaccine alone. In fact, few countries managed to eliminate brucellosis from endemic cattle herds only by culling positive animals, without complementary measures (Zhang et al. 2018). Our results confirm this difficulty.

Table 3. Incidence of abortion in beef cows after immunization against brucellosis with RB51 vaccine once (G1), twice (G2) or in the control group (G3).

Group	**Abortion**		
	Farm 1	**Farm 2**	**Combined**
G1 (1x vaccine)	1.7% (3/176)	2.7% (4/146)	2.2% (7/322)
G2 (2x vaccine)	3.0% (4/135)	2.1% (3/144)	2.5% (7/279)
G3 (Control)	4.3% (6/138)	5.0% (7/139)	4.7% (13/277)

In our study we observed no difference in the percentage of abortion between immunized (once or twice) or non-immunized cows. This result corroborates the previous findings from Barbosa et al. (2017), who demonstrated the safety of vaccination of cattle with RB51 at different stages of pregnancy.

Reproductive efficiency outcomes from Farm 1 are shown in Table 4. There was no difference (P>0.05) on the calving to first AI interval, on the number of inseminations per conception, or on the percentage of abortion. However, unvaccinated cows (G3) presented a greater number of days the animals remained open, when compared to vaccinated cows (G1 and G2).

Table 4: Reproductive efficiency of cows vaccinated once (G1) or twice (G2) with RB51, and from the control group (non-RB51 vaccinated), on a beef farm that adopted FTAI throughout the year.

Group	**N**	**Calving-first AI[1]**	**AI/ Conception**	**Days Open**
G1 (1x vaccine)	176	36.2±7.8[a]	1.91±0.24[a]	79.7±14.2[b]
G2 (2x vaccine)	135	35.4±7.2[a]	1.83±0.22[a]	77.2±13.7[b]
G3 (Control)	138	39.1±8.9[a]	2.25±0.4[a]	85.5±15.9[a]

[1] Means in days

[a,b] Values followed by different superscripts, on the same column, differ (P<0.05)

There was no difference (P>0.05) in the interval from calving to the first AI or on the percentage of abortion among groups. However, the pregnancy rate at the end of the breeding season was greater (P=0.0376) in cows from G1 than from G3, although neither were different (P>0.05) from G2, as shown in Table 5.

Table 5: Reproductive efficiency of cows vaccinated once (G1) or twice (G2) with RB51, and from the control group (non-RB51 vaccinated), on a beef farm that adopted a breeding season.

Group	N	Calving-first AI[1]	Pregnancy rate
G1 (1x vaccine)	138	41.2±9.6[a]	89.1%[a]
G2 (2x vaccine)	137	40.5±10.2[a]	85.0%[ab]
G3 (Control)	141	43.1±9.8[a]	80.1%[b]

[1] Means in days

[a,b] Values followed by different superscripts, on the same column, differ (P<0.05)

Altogether, the endpoints evaluated on the farms enrolled in the current study suggests an improvement in reproductive performance subsequent to vaccination against brucellosis, despite the differences in the breeding systems adopted. In fact, the reproductive problems caused by brucellosis and their consequence on herd reproductive performance are well known (Bernués et al. 1997). In particular, abortion during the last trimester of pregnancy and consequent high incidence of retained placenta are expected to have a more immediate impact on parameters such as pregnancy rate or days open than on calving to first AI or AI per conception, as observed here. However, it is likely that a statistically significant effect would also be observed on most fertility indexes if a greater amount of data were analyzed.

Conclusion

The immunization of cows strain 19 calfhood vaccinated against brucellosis with a single dose of a rough strain RB51 vaccine was effective in preventing new infection of beef cows managed in an extensive regime, without increasing the occurrence of false positives. Immunization with Strain19 only was less effective. Moreover, boost vaccination with RB51 improved reproductive performance of herds with endemic brucellosis.

Thanks and recognition

Pereira, G.H.S. MV, MsC. and Pereira, J.R. MsC. and (Biotran LTD - Brazil) for investigation and data compilation; Viana, J.HM. MV, PhD (Embrapa – Cenargen - Brazil) for formal analysis, writing – review & editing.

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Bovine Brucella Vaccines in Latin America

Luis Ernesto Samartino

Introduction

Bovine brucellosis is an infectious and contagious disease transmitted from animals to humans, constituting one of the most widespread zoonoses worldwide and fitting perfectly within the term "one health".

The control or eradication of bovine brucellosis is based on epidemiology, diagnosis, vaccination, and the slaughter of positive animals.

Briefly, we will describe some basic concepts of bovine brucellosis. *Brucella abortus* (*B. abortus*) is a Gram negative bacteria capable of multiplying in the rough endoplasmatic reticulum (RER) of trophoblasts and macrophages, which is a unique phenomenon within the bacteriological world. The main form of infection is through the digestive route by ingestion of food contaminated with vaginal secretions and milk from infected females. The incubation period of brucellosis is related to the physiological state, being always shorter in the pregnant animal. The main sign of the disease is abortion, mostly in the last third of pregnancy; meanwhile infection often occurs from 15 days before abortion/birth until 4 weeks following pregnancy. Experimental studies showed that up to $1x10^{14}$ *Brucella* per gram can be excreted from the placenta, which demonstrates the seriousness of abortion. It should be noted that in a normal birth of an infected animal, similar amounts of bacteria can be excreted as in an animal that has aborted. For this reason, although a third of infected bovine females DO NEVER abort, they are equally dangerous in terms of contagion to other animals, mainly at the time of birth. Retained placenta frequently accompanies abortion and/or birth of animals with *Brucella* infection. Colostrum and milk also carry *Brucella* with intermittent shedding.

Farm animal infection and disease control

The primary cause of infection into a farm is due to the entry of infected animals and/or incubating the disease. Therefore, the health status of the herd must be always known. When positive animals are detected, NO animals should be introduced into the existing herd, as there is a high probability that there are serologically negative animals in the incubation phase and therefore they are not detected. The adjacent farm should always be considered, since there is a risk for a farm eradicating the disease that there are adjacent herds infected with brucellosis. This is a community disease and must be fought together; that is where veterinarians and state authorities must proceed to take measures so that its control is comprehensively carried out. We know that the economic factor plays a predominant role for the producer, who must eliminate the positive animals. Obviously, keeping those animals results in a perpetuating factor of the disease. These concepts are essential to understand the spreading of the infection in farm herds, and, eventually, the vaccination and revaccination strategies.

Diagnosis

The definitive diagnosis is the isolation of the causal germ, in this case *B abortus*. It is recommended that each country knows which biovars are present in their territory. For example, biovar 1 is present throughout the Americas, however, some countries like Argentina, biovar 2 is also present, which is more pathogenic for humans and causes severe abortion storms and also, birth deaths. Molecular techniques are also available through PCR.

Nevertheless, serological tests mainly have been used since the end of 19th century for control and eradication programs. Numerous serological tests have been developed since the old tube agglutination test to screening tests, plate tests, like Rose Bengal or Buffer Plate Antigens, and confirmatory tests like 2 mercapthoetanol/rivanol, or later, primary binding test such as ELISA and/or Fluorescent Polarization. In addition, Milk Ring Test and Indirect ELISA are widely used in the dairy cattle. Very important, we must always recall that all these tests detect the "O chain" of the lipopolysaccharide (LPS) of *B. abortus*, which is immunodominant and induces antibodies against this bacterial antigen (Figure 1). So, we have enough tests to identify the disease and all of them are used in LATAM (by different strategies).

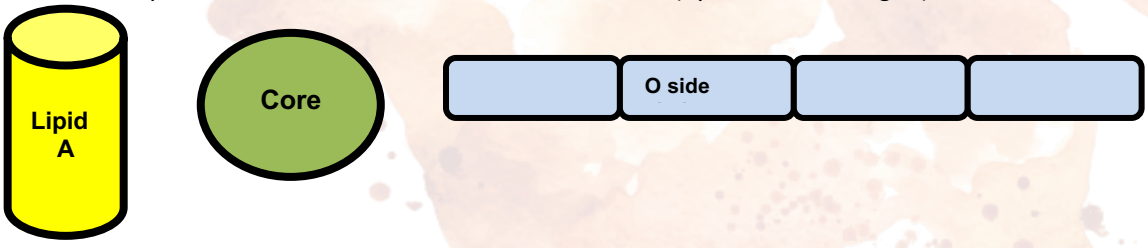

Figure 1. Scheme of the structure of *Brucella* Lipopolysaccharide (LPS). LPS has 3 parts: a lipid A part, a central part (core) and the "O" chain, which is the inducer of the antibodies that are measured in conventional serology.

Vaccines for cattle

We have briefly described the pathogenesis of bovine brucellosis and its diagnosis. However, prevention is a fundamental aspect for the control and/or eradication of this disease. Currently, there are two vaccines in the world recognized by the World Organization for Animal Health (WHO) for the prevention of bovine brucellosis: strain 19 (S19) and strain RB51.

We must remember that the protection provided by vaccines against brucellosis is due to cell-mediated immunity (CI), with little or no importance for protection mediated by antibodies (humoral immunity).

Strain 19 is a naturally attenuated Gram-negative *B. abortus.* It has been known since the 1930s, initially applied in the United States (USA). The immunity granted is relative and ranges between 65 and 70%. S19 has its complete LPS, meaning that includes O chain; thus, it must induce antibodies (Figure 2). In most of the countries, female calves are vaccinated between 3 and 8 months of age with strain 19, meanwhile, adult vaccination is NOT recommended due to the induction of serological titers that confuse the diagnosis. Although it has been known for many years that strain 19 does not protect "for life", even today in some countries, such as Argentina, it is the only approved vaccine. However, several factors may influence the previous statement, such as the prevalence of the current disease, or the persistence of outbreaks on already vaccinated herds. These factors are independent of the "quality" of the vaccine, which it rather fulfills its protective task until the bacterial load present in the herd is large enough to break the immunity. Due to similar situations, in USA during the 1970s, when it was observed that the prevalence of the disease in cattle had decreased, but far from being controlled, it was decided to implement the adult vaccination program with a reduced dose of Strain 19. The reduced dose had one logarithm less concentration than the usual dose, and therefore the serological titers were expected to disappear before 6 months after vaccination.

Meanwhile, during the 1980s, a live vaccine was developed at Virginia Tech University (Virginia, USA) by Dr. Gerhardt Schurig, This strain, named RB51, was, after all, a vaccine composed of a live rough strain of *B. abortus*. It should be noted that this rough strain has an LPS that lacks the O chain, therefore it does NOT induce the production of antibody titers that can be detected by conventional diagnostic methods and, therefore, facilitates the identification of sick animals without risk that "false positives" are condemned (Figure 2).

Different studies carried out made it possible to verify that vaccination of calves (4-10 months of age) with 1 x 10^{10} CFU (Colony Forming Units) of strain RB51 induces immunity against challenge with experimental *Brucella* similar as induced by the strain 19, generating protection against disease and abortion. Besides, it was stable (did not revert to pathogenicity) and DID NOT induce serological responses which interfere with diagnostic tests, even if the animals received more than one dose.

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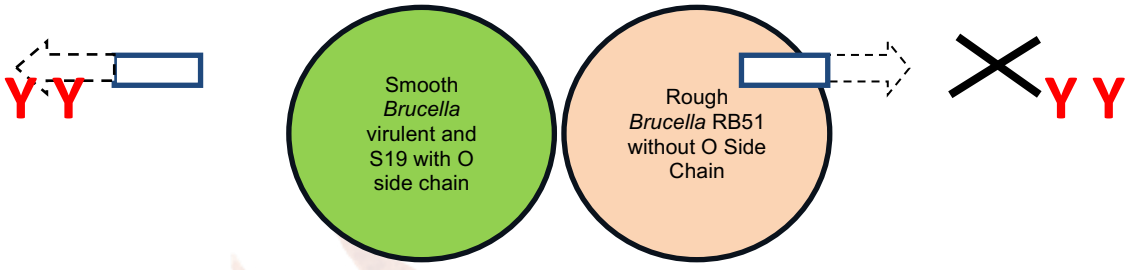

Figure 2. Scheme of the interaction of the "O" chain of the LPS molecule found on the surface of smooth virulent *Brucella* and also in the S19 strain

Consequently, after the success of these preliminary trials, the USDA (USA) carried out several studies in cattle and demonstrated that the protection provided by the RB51 strain was similar as provided by strain 19. Therefore, in 1996, the USDA adopted this vaccine to replace strain 19, initially for application in calves from 4 to 12 months of age by subcutaneous route, improving its control and eradication program. It should be considered, contrary to what it is frequently thought, that at that time, brucellosis was still present in some states of the USA and even endemic in wild animals (e.g. buffalo, caribou etc.).

Brucella vaccines for cattle in Latin-American (LATAM)

Although it is not the objective of this manuscript to discuss bovine brucellosis control programs in the LATAM region, we describe some general concepts adopted by LATAM countries where differences by using the vaccine were noticed.

All Veterinary Services in LATAM have developed official bovine brucellosis control and/or eradication programs. All of them began around mid 20th century and included brucellosis surveillance in order to detect and eliminate infected cattle, and "official vaccination campaign" by using S19 of 4 to 8/12 months according to country regulations.

Such programs are briefly described, Colombia sets an example in 1959, vaccinating female calves between 4 to 12 months but in 1969 modified the regulation and vaccinated calves of 4 to 9 months. Uruguay started to vaccinate with S19 in 1953 and Argentina in 1958 mainly in dairy herds, however in this last country S19 vaccination is mandatory to all female calves between 3 to 8 months since 1982 till today. The strategy of the control programs, as example, includes compulsory S19 vaccination like in Argentina and Uruguay in all their territory Argentina in 1993 vaccinated cattle between 3 to 10 month-old, but a new resolution on 1999 turns back to 3 to 8 month-old. Uruguay used to apply the vaccine to all female calves between 3 to 6 months old. On the other hand, "a select vaccination program" is chosen according to prevalence of bovine brucellosis on different regions within the country.

Chile is a very interesting example. It started the brucellosis program in 1975, using S19 voluntary vaccination in south central region, but, in other areas, only used serological detection and slaughter of cattle. Brazil may be the best example of variation of the program according to different conditions within the country. Obviously, the enormous size and different characteristics of the regions were decisive factors. Although S19 was introduced in the middle of 20th century, southern states had a more intense program of vaccinations than northern state. In 1976, another national program was issued based mainly on the S19 voluntary vaccination of heifers, detection of infected herds and voluntary culling of positive animals. However, the program was never fully implemented, and the epidemiological situation remained stable with high prevalence of the disease in the regions with the highest livestock populations. Programs of compulsory S19 vaccinations in Mato Groso, San Pablo, Minas Gerais, Rio Grande do Sul, Santa Catarina are examples during the 80s/90s of the last century.

Mexico has a large history of vaccination programs with different success during last century. Although most of the Central American countries adopt alternative strategies of S19 vaccination (sometimes voluntarily), they never reached a high percentage of vaccinated animals. Again, all the programs mentioned above include the elimination of the positive animals.

Due to the frequent appearance of brucellosis outbreaks in vaccinated adult animals, some countries implemented adult vaccination programs with a reduced dose of Strain 19, reflecting the program applied in the USA. A similar program was also implemented in the 1980s in Mexico and Chile. However, although the vaccination of adult animals decreased brucellosis in these places, there was the problem of false positive animals, which were NOT able to reduce their level of antibodies and were wrongly sent to slaughterhouses. In Argentina at the end of the 90s, adult animals were revaccinated with "non-controlled reduce doses of S19" without knowing the correct number of bacteria contained in such dose (precise number of colony-forming units), thus, cattle presented permanent serological titers or, even more serious, did not achieve the main objective of controlling abortions and infection. So, this useful tool was misused, since there were no laboratories producing this vaccine even when the regulations of those years allowed it (SENASA resolution 1269/93).

We know that the limitation of S19 due to the induction of antibodies meant that national programs began to control the disease from 22/24 months of age. Over the years, with the drop of the age of breeding (13 to 14 months mainly in dairy heifers) a vaccinated animal at the regulatory age should give birth before 24 month-old. Thus, if such animal became infected, not only she would spread *Brucella* but also can abort before that age, which is why the control age of the animals became a serious drawback. This forced the elaboration of another program, (like in Argentina, for example) lowering the control age to 18 months old but caused another inconvenience: some animals vaccinated to the limit (8 months) or more appeared with serological titers suspected of being S19 vaccinated but cannot be distinguished from those that were actually infected.

With the arrival of the 21st century, another vaccination strategy started in LATAM: the introduction of the RB51^{tm} vaccine to bovine brucellosis programs. Although in some countries (Mexico and Chile for example) this vaccine was introduced in the late 90s, the majority of the remaining countries introduced the abovementioned vaccine in the 21st century. All of these programs have the main objective of reducing the prevalence of the disease to levels that allow it to be eradicated from the "national" territory in accordance with the Prevention, Control and Eradication Program of Bovine Brucellosis.

We briefly describe here some examples of the new different vaccination strategies where RB51[tm] was included as a new tool within the necessary complementary measures to eradicate the disease. Chile, again, is a good example of this, following exactly what happened in the USA. In 1997, Animal Health authorities (SAG) prohibited the use of S19 and introduced the use of the RB51 to be applied between 6 and 8 months of age. Furthermore, the vaccination of adults was possible when the epidemiological situation it was suitable, mainly in quarantined properties. This strategy was intensified when a complete disease eradication plan (different strategies depending on region) was initiated through the SAG in December 2004 with the following vaccination schedule:

A. Vaccination in non-infected herds or with a low level of exposure to *B. abortus infection*. A vaccination program must consider: - Vaccination of all female cattle when they have reached 5 months of age. - Revaccination of females prior to the mating period. - Vaccination of all females entering the herd.

B. - Herds Infected or Exposed to a High Risk of Infection by *B. abortus*. Vaccination of all female bovines: calves, pre-breeding heifers and cows, including pregnant ones, will be carried out with strain RB51. Prior to its application, the Accredited Veterinarian must inform the owner of the herd of the risk of abortions due to strain RB51, and must state in writing his agreement with the decision. Currently, in addition to a surveillance program throughout the territory, farms with confirmed infection are subjected to farm quarantine, whose measures include restriction of movement and rapid elimination of infected cattle, periodic checks to detect new infected animals and vaccination with RB-51 from exposed susceptible animals. Chile has a large zone free of bovine brucellosis; however new areas of infection still appear, which highlights the importance of strict control and frequent vaccination.

Uruguay has a similar vaccination strategy, with some interesting differences. In this country, in 1996, the systematic vaccination of cattle with the Strain 19 vaccine was forbidden due to the absence of brucellosis outbreaks continuing the eradication program without the use of vaccine. However, in 2002, due to the appearance of important outbreaks of brucellosis in a beef cattle region, the application of the strain RB51 was authorized for the infected area. Besides, in 2003, another important outbreak of brucellosis appeared in a dairy area, applying RB51 again in the region. In 2005, mandatory vaccination against bovine brucellosis with the RB51 vaccine was established for all females over four months of age, not only in the those affected regions but also in those epidemiologically related areas established by the country's Animal Health Directorate. The vaccination strategy with this vaccine was similar to that implemented in Chile. Actually, the prevalence of brucellosis in cattle in Uruguay is less than 1%. These countries continue to use only the RB51 vaccine for the control of bovine brucellosis.

We can see another different strategy in Argentina with The National Plan for the Control and Eradication of Brucellosis in Bovines according to resolutions established in 2019 and their amendment in 2021 of SENASA. The activities under the Program include mandatory vaccination, in 100% of calves from 3 to 8 months of age with the S19 vaccine, simultaneously with the foot-and-mouth vaccination campaigns. However, the last amendment of 2021 mentioned above include the possibility of using “other vaccines” just for adult cattle after evaluation of such strategies. In other words, the country keeps the same criteria since 1982.

In Central America, Costa Rica, Guatemala and Dominican Republic, allow both vaccines S19 and RB51, both can be used in calves however only RB51 can be used in adults females. In 1999 Costa Rica vaccinated mainly with RB51 with emphasis on dairy cattle. In Colombia where S19 is still applied in calves, the program against bovine brucellosis is based on ICA Resolution of 2013. The vaccine is applied between 3 and 9 months of age using S19 or Strain RB51 and, between 9 and 15 months of age, to those calves first vaccinated with RB51 and reinforced with the same vaccine. This is done together with the foot-and-mouth vaccination cycles that are carried out twice a year throughout the national territory.

In Ecuador, the health authorities (AGROCALIDAD) issued a resolution in 2016, indicating vaccination with strain 19 for cattle between 3 and 6 months of age, also allowing vaccination with RB51. However, only is allowed in cattle over 8 months old and also in those farms where *Brucella* infection was detected. In addition to eliminating the positive ones, RB51 need to be applied to all negative animals. Bovine brucellosis program in Bolivia (SENASAG) indicates mandatory vaccination either with S19 or RB51 for cattle between 3 and 8 months of age with emphasis in two departments, Cochabamba (dairy cattle) and Beni (beef cattle) where most of the herds are concentrated.

Brazil launched a new national brucellosis and tuberculosis program in 2001 with compulsory vaccination of heifers of 3 to 8 months only with S19 but, again, strategy was different according to the states. Vaccination programs within southern states were more consistent than other regions in the country. In 2007, the RB51 strain was approved for vaccinating female cattle older than 8 months of age and its use is at the discretion of the veterinarian. A normative Instruction in 2017 issue by the National Program

indicates that female cattle between 3 and 8 months can be vaccinated with the S19 vaccine or the RB51 vaccine based on the decision of the producer. RB51 remains authorized for use in unvaccinated adult females meanwhile the use of the S19 vaccine in females over eight months of age is banned. Besides, unvaccinated calves from 3 to 8 months of age must have their vaccination status regularized, using the RB51.

Paraguay, on the other hand, maintains the application of S19 in calves based on a modification of the vaccination strategy in 2017, and it's National Service for Animal Quality and Health (SENACSA). It introduced the program with systematic and mandatory vaccination of young females between 3 to 8 months of age using the S19, and mandatory RB51 vaccination at mating and/or insemination. So these females begin their period of reproductive activity with two vaccinations. Furthermore, during control of brucellosis infected farms, the vaccine to be used in adult females (cows and heifers) is only RB51. Interestingly, this program had a paragraph that says the following: "As the program progresses and there are results of decreased prevalence, it is proposed to study the exclusive use of the RB51 vaccine." In 2023, SENACSA together with the Animal Health Services Foundation (Fundassa) presented the results of a sampling study indicating a 50% reduction in the prevalence of bovine brucellosis in the last 7 years of vaccination. This sampling was to measure the effectiveness of the vaccination work, and they found encouraging results that bovine brucellosis had a 50% decline in prevalence in the last 7 years of vaccination. At the end of 2023, Animal Health authorities issue a resolution indicating that in 2024 the only vaccine allowed in the country to be used in cattle and buffalo will be RB51.

Conclusions

To summarize, most of the LATAM countries use S19 and/or RB51 either in calves and/or adults by using different strategies today. Therefore, as examples, Mexico, Costa Rica, Guatemala, and Ecuador use a larger percentage of RB51 over S19; Colombia, and Dominican Republic vaccinate mainly calves with S19 and adults with RB51, Brazil can use both vaccines according to the states, meanwhile Panama and Nicaragua do not use vaccines. Argentina uses only S19 for calves and Chile, Uruguay and, recently Paraguay, only utilize RB51.

Control and eradication of bovine brucellosis must involve extensive diagnoses of infected herds by high quality serological assays, mandatory culling of the serological positive animals (including compensation actions if possible) and restriction in the traffic of animals from infected areas to free areas. However, vaccination is the most important strategic tool available to prevent the disease. The vaccination campaigns in LATAM have never been adequately adopted to raise the herd immunity necessary to decrease the number of susceptible animals for control programs. We have the tools, we have vaccines, we performed different strategies for using them, so we must use massive vaccination in cattle to prevent the disease, to increase herd immunity and, most importantly, to prevent a major zoonosis.

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These references are part of the literature revised to bring into this abstract. Of course brucellosis programs with emphasis in vaccination from all countries of LATAM were also examined.

PRACTICAL STRATEGIES FOR THE ELIMINATION OF BOVINE BRUCELLOSIS IN MEXICO.

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BACKGROUND.

The Laguna Region is the most important area in Mexico in milk production, with a volume close to 2,570 million liters in 2020 (21% of national production), with around 500,000 cows. La Laguna dairy farmers are characterized by having herds ranging from more than 500 to 6,000 (or more) cows in production, with Holstein cattle. With genetics originally imported from the United States and Canada, but with remarkable epigenetic impacts; and with average yields of 30 to 45 liters per cow per day.

Even before its consolidation as a dairy basin, the Laguna Region faced the challenge of eliminating bovine brucellosis, derived from the increase in cases of Malta fever in the milk-consuming population. Above all, because the state government of those years required ranchers to do something concrete and fast to contribute to the reduction of this public health problem. This forced the organized dairy farmers of the region to, first, integrate a credit union that would facilitate the obtaining of economic and financial resources and, later, to install a pasteurization cooperative plant that would guarantee the safety of the milk consumed by the consuming population.

ECONOMIC AND HEALTH SITUATION

Notwithstanding the above, and considering that pasteurization guarantees the safety of milk for the consuming population, until 2013 it had not been possible to achieve the objective of eliminating brucellosis in the bovine population that supplies the main pasteurization companies in the region. Reaching a regional prevalence of 35% of herds, herds with up to 65% of internal prevalence.

For this reason, and with the intention of being at the forefront of the global milk markets, the most important Cooperative that collects milk in the region (and in the country) made the decision agreed with the farmers, and in technical consultation with the Veterinarians both in private practice, as members of the Committee for the Eradication of Bovine Tuberculosis and Brucellosis. to eliminate brucellosis in milk-supplying cows. And so it was that the ambitious goal of eliminating bovine brucellosis by 2018 was set.

IMPLICATIONS.

This implied the harmonization of procedures, the unification of technical-veterinary criteria and the group assumption of a collegiate and livestock union decision. Three fundamental premises were established: The application of a basic general scheme of control and elimination of the disease in each and every one of the herds in the region, with specific adaptations in each herd according to its environment and internal management.

The unrestricted acceptance of the results of accepted diagnostic tests. To this end, it was essential to have proven, validated, rapid execution, economical, sensitive, and specific diagnostic techniques.

As well as the rational use of biosecurity and preventive medicine schemes.

TOOLS USED

The diagnostic technologies accepted for use in this regional control program were, individually, the fluorescence polarization assay (FPA). And as a test of epidemiological surveillance in herds, the indirect ELISA test. It is worth mentioning that neither of the two tests, although authorized by the World Organization for Animal Health for some time, and which were validated since the end of the last century in Mexico to be used in the campaign, were not tests consigned to the official Mexican regulations. However, given their

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characteristics of diagnostic plausibility, efficacy and efficiency, as well as their performance in massive control and surveillance activities, favored the decision to use them routinely. In this way, a basic scheme of action was established where the frequency and regularity with which the diagnostic "filters" would be made, the areas in which they would be done, as well as the criteria to be taken into account for the management of the animals that tested positive, were established.

On the other hand, derived from the diagnostic technology used, it was necessary to have a vaccine that would not interfere with the diagnostic tests to be used. (Just as it was with strain 19 previously.) For this reason, and considering that it was accepted as an official vaccine in Mexico, the exclusive use of the vaccine, RB51 of *B. abortus* was accepted to be used in vaccination and revaccination programs for the prevention of brucellosis in the region. A vaccination schedule was proposed, starting the application of the dose to calves from 4 months of age, application of a booster 6 months later and another vaccination to the AI. Considering the experience that had been accumulated since the nineties in the region with the use of this vaccine, making the application of the RB51 vaccine of *Brucella abortus* the pillar of preventive schemes.

With respect to the other preventive medicine and biosecurity measures, the segregation or elimination of animals that tested positive for diagnostic tests was established as a management premise. Likewise, they assumed the end of the productive cycle of these animals and did not include them in a new reproductive cycle, canceling their insemination.

Another essential point was the management of the rearing from the birth of the heifer. Giving colostrum only from known negative mothers, or colostrum substitutes or pasteurized colostrum. The latter being the most demanded technology.

Likewise, although it is not an orthodox recommendation, the systematic application of cresolic disinfectants in risk areas (pens of animals at calving, for example) was established with very good results.

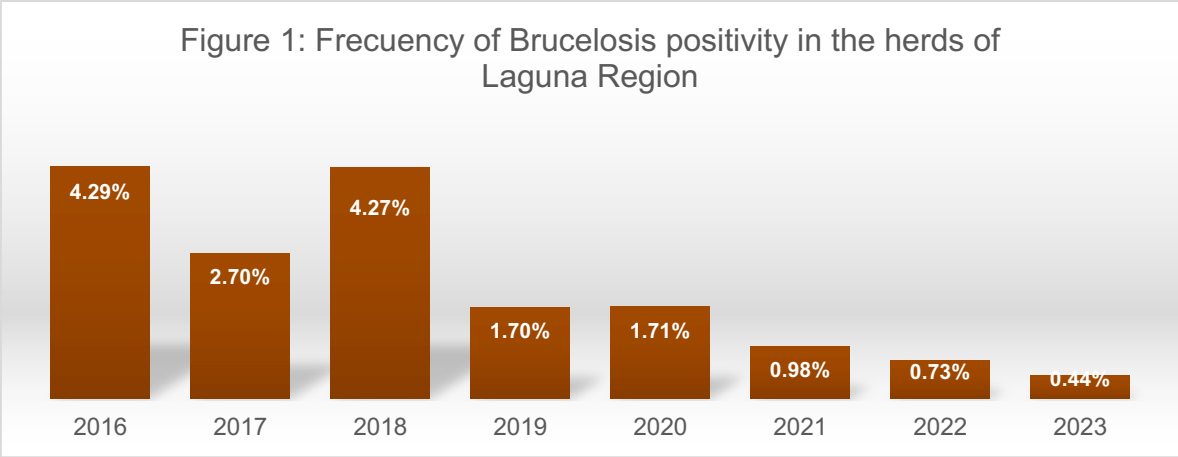
In this sense, the practice of giving the calf pens the surplus feed that had not been consumed by the adult animals (a practice deeply rooted for economic reasons) was also eliminated in several herds.

Undoubtedly, one of the determining points was, in addition to the political-business decision, the assumption of the financial implications of the project. The absorption of the costs of diagnosis, vaccination, preventive medicine schemes and biosecurity by the producer is essential. Added to this was the financial cost of the disposal of productive and potentially productive animals (heifers), which tested positive for diagnostic tests.

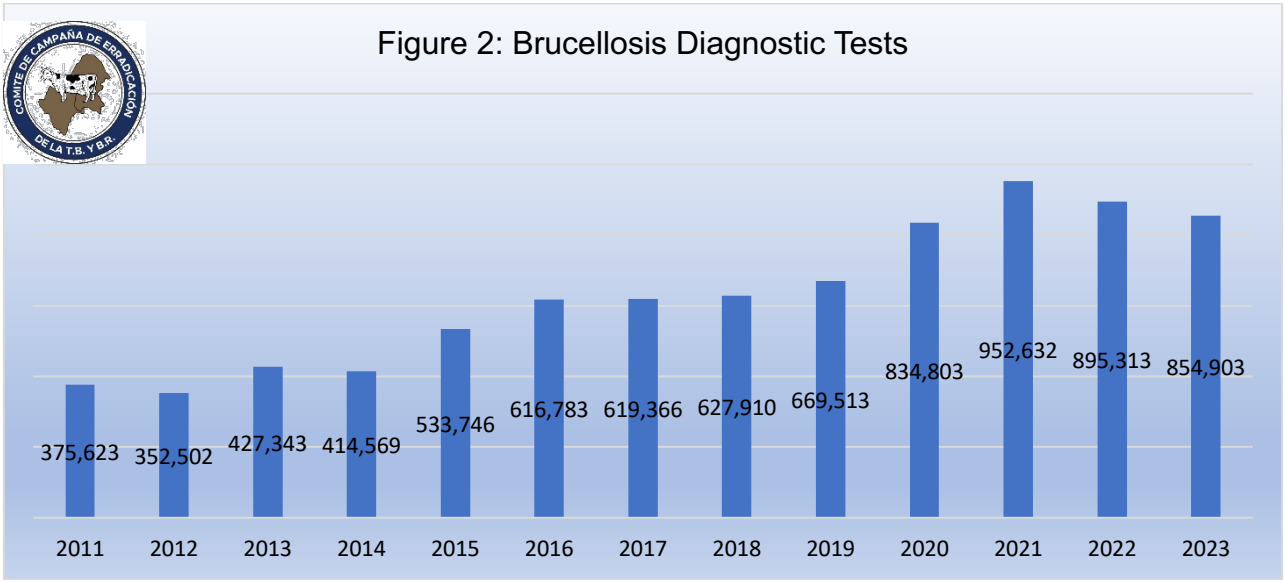
But undoubtedly the most impactful decisive factor was the support offered (and situated) by the main pasteurizing cooperative company in the region (Lala®), to pay an incentive for each liter of milk that came from negative animals to the disease under this diagnostic and control scheme. This was the factor that triggered the massive participation of each dairy supplying stable.

ACHIEVEMENTS.

Figure 1 shows the evaluation of regional serological frequency:



Although it is accepted that when the prevalence of a condition in a population is lower than the rate of false positives (in the case of FPA it is considered 3.1%), it is more likely that the "positivity" found in the tests will be qualified as "false positives" than the presence of the condition, surveillance and monitoring activities of both animals and herds are maintained. as well as the vaccination program.
Figure 2 shows the number of diagnoses made per year in the Committee for the Eradication of Tuberculosis and Brucellosis of the Laguna Region.



FINAL THOUGHTS.
The regional brucellosis elimination program in the Lagunera Region is considered successful. The combination of efforts and wise decisions determined the effectiveness and efficiency of the actions, as well as the commitment of those involved, industry, producers, veterinarians and laboratory personnel.
The success of the program also makes it possible to establish as premises for an effective control program: Knowing the diagnostic test used, its interpretation, scope and objectives. It is also essential to accept the result of the test without restriction, with the consequent decision to carry out pertinent actions based on the result, such as isolation or elimination of the animals that test positive. On the other hand, it is essential to have a reliable vaccine and also to know its management, scope and limitations. And understand that vaccination alone

does not solve problems, but must be part of a whole model of biosecurity and preventive medicine on site.

RECOGNITION.

The work of the Veterinary Medical Staff and Laboratory Technicians of the Committee for the Eradication of Bovine Tuberculosis and Brucellosis of the Lagunera region Mexico is recognized, for the achievement of the objectives of elimination and significant reduction of the prevalence of brucellosis in La Laguna.

Calving & calf care management

1. How accurately can we predict calving?

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More than a dozen indicators of impending parturition have been tested to develop calving alarms. Commercially available calving prediction devices focus on monitoring dam activity, body temperature, tail elevation, and fetal expulsion, though other physiological processes can also be monitored (Mee, 2021). Of activity monitoring devices, a combination of rumination time and posture changes appears to offer the best prediction accuracy, but the cost may be an issue for some breeders not already using precision livestock farming (PLF) technology. Of fetal expulsion devices, vulval lips separation devices appear to offer the best prediction accuracy, but these must be attached by a veterinarian. Of tail elevation devices, tail-mounted accelerometers appear to provide the best prediction accuracy, but issues with tail injuries and no/false alerts may occur (Umaña Sedó et al., 2024). Of thermosensors, intra-vaginal devices appear to provide the best prediction accuracy, but the animals must be restrained, and the devices must be inserted hygienically in the vagina (Szenci, 2022).

Currently, there are no published studies comparing all commercially available calving prediction devices together, so we must rely on studies where one device is compared with breeder observations (Szenci, 2022) or where more than one device is compared on the same animal (Horváth et al., 2021). Such devices vary in accuracy and cost, and sometimes, more importantly, their suitability for use on cows close to calving.

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2. Assessment of neonatal vitality - To decrease the prevalence rate of stillbirth

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Stillbirth (perinatal mortality) is the death of a mature fetal calf with longer than 260 days of gestation during calving or within 24 to 48 hours of postnatal life. Since a dairy farm's profitability greatly depends on the calves' rate of being born alive and reared to adulthood, if the stillbirth prevalence rate is higher than ≥3% on a given dairy farm, then the farm management has a critical task of decreasing its prevalence rate.
Before starting any obstetrical assistance in anterior or posterior presentation, it is essential to emphasize that we must be 100% accurate in our diagnosis, whether the fetus is still alive or not, to select the most appropriate method for our obstetrical assistance. In doubtful cases, we must use ultrasonography, a pulse oximeter, or measure acid-base balance or lactate concentration to confirm our diagnosis. In cases of severe asphyxia, it is better to perform a Cesarean section to save the fetal life than doing traction, even when the duration would be less than 60 seconds. After calving, several vitality scores may help us evaluate the vigor of a newborn calf. Originally four different clinical signs were recommended to assess the well-being of a newborn calf. Later, five different clinical signs or even more or less were recommended to evaluate the vitality. However, several attempts have been made to create a practical tool to assess newborn calf vitality, a helpful tool that can be used on farms with ease and high accuracy is still missing. Measuring acid-base balance or lactate concentration may increase the accuracy of our diagnosis (Szenci, 2023).
There are already several diagnostic methods to evaluate the clinical signs of vitality during and after calving or to measure acid-base balance or L-lactate concentrations, as well as to use ultrasonography to detect heart rate or pulse oximetry to measure continuous fetal/neonatal

oxygen saturation of arterial hemoglobin and heart rate already on dairy farms. All these methods may contribute to recognizing and eliminating threats to the vitality of the fetal/neonatal calf in time. From this point of view, farm management has the critical task of selecting and applying the methods that can be used most effectively under the given circumstances—considering current economic aspects as well. All these methods can help prevent the damage caused by dystocia, which often contributes to fetal/neonatal mortality. It is essential to emphasize that we must decrease the prevalence rate of dystocia to decrease the occurrence of severe asphyxia. If we cannot avoid the development of asphyxia, we must take care of adequate treatment to reduce its losses. Therefore, this workshop partly focuses on the diagnostic possibilities and limitations of evaluating fetal and neonatal vitality in dairy practices.

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3. Colostrum management: Setting the calf and producer up for success

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Achieving high levels of passive immunity is critical to reducing the risk of common calf-hood diseases, such as diarrhea and respiratory disease (Crannell and Abuelo, 2023). Passive immunity can be successfully transferred by feeding an adequate quantity of high-quality, clean colostrum quickly after calving (Renaud et al., 2020). Although new research has identified tweaks that can further enhance colostrum management on dairy farms, the rates of failed transfer of passive immunity remain high. Therefore, it is critical to understand the main barriers to change for calf management on dairy farms and the critical role veterinary practitioners can play (Kaske and Bleul, 2021). A major barrier to improving calf care is a need for knowledge regarding best management practices. As veterinarians are highly trusted to be a primary source of knowledge for dairy farmers, and

veterinary engagement in calf management can have a tremendous impact. Additional barriers include the prioritization of the milking herd, meaning producers value simple, time-efficient, and economical solutions to calf care challenges. Other barriers include farm infrastructure and challenges in employee training (Wilson et al., 2021). Beyond barriers, it has been identified that producers are motivated by social norms, intrinsic pride, and an obligation to provide good calf care. This may explain why benchmarking is an important tool to motivate producers (Wilson et al., 2023). Veterinarians can play a key role in overcoming these barriers and managing programs to motivate change in calf care practices. Colostrum management has been consistently identified as a major influencer of calf health; however, uptake of optimal colostrum management strategies has been low. Understanding barriers and motivations for change can aid in improving the rates of passive immunity. Veterinarians play a major role in driving on-farm change and can serve to deliver up-to-date information on colostrum management to farmers.

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