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DETECTABLE IMMUNOLOGICAL RESPONSE IN *BRUCELLA ABORTUS* S 19 VACCINATED HERDS OF CATTLE IN VILACHCHIYA VETERINARY RANGE, NORTH CENTRAL PROVINCE, SRI LANKA

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SUMMARY: Brucellosis a zoonotic disease caused by Gram-negative, intracellular, coccobacillus. Brucellosis, an economically important disease in livestock with zoonotic implications has been reported worldwide. It is transmitted via contaminated animals and their products. Clinical signs of animal brucellosis are abortions, reduced fertility, retention of fetal membranes, endometritis, and substantial decline in milk production over an animal's lifespan. Orchitis, epididymitis and chronic hygroma had been reported in bulls. The current study focused on to studying the detectable immunological responses to Brucella S 19 live attenuated vaccine using serological tests in cattle of dry zone farms. A questionnaire-based survey was carried out in selected farms with and without a previous history of abortion respectively. Animals that were in 1st to 5th lactation in the 18 farms were selected for screening by Rose Bengal Plate Test (RBPT) and positives were confirmed by Complement Fixation Test (CFT). Three farms each with and without history of abortion were selected for Brucella S19 vaccination from the RBPT and CFT negative farms. Sixty non pregnant heifers over 6 months were selected, and 1ml each of S19 vaccine was given subcutaneously to 30 animals while 30 animals were kept as controls. The serum samples were collected and screened for Brucellosis by RBPT and CFT at 2, 4, 6, 8 and 10 weeks of post vaccination. The second week after the vaccination, 71.42% of the animals showed over 1:4 CFT levels while 28.57% of them did not show any antibody titres for Brucellosis in the Brucella endemic farms. In Brucella non-endemic farms, 68.75% of animals showed over 1:4 CFT titre at 2 weeks after the vaccination. However, 31.25% of animals did not show any CFT titre. No persistent and detectable antibody responses were found 10 weeks post vaccination in heifers of endemic and non-endemic herds. The titers of CFT indicate IgG or IgM humeral response, cell mediated immune response of live attenuated vaccine has not been monitored in the study. Thus, seropositivity after the 10 weeks post vaccination may indicate an infection of Brucellosis since no antibodies from vaccination were found. Further investigation with highly sensitive tests such as ELISA is recommended to have a better insight into this aspect in the future.

KEY WORDS: *Brucella abortus*, Brucella S 19 vaccine, RBPT, CMT

INTRODUCTION

Brucella is a Gram-negative, intracellular, aerobic coccobacilli with a diameter of 0.5-0.7 μm and has 0.6-1.5 μm length (He, 2012; Khurana *et al*., 2021). The bacterium is shown to be partially acid fast, positive for oxidase, catalase, nitrate reductase, and urease test (Khurana *et al*., 2021). According to the antigenic variation and differences in the primary host, Brucella species have been classified as; *Brucella melitensis* in sheep and goats, *Brucella abortus* in cattle, *Brucella suis* in pigs, *Brucella ovis* in sheep, *Brucella canis* in dogs, *Brucella ceti* and *Brucella pinnipedialis* in marine mammals, and

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Brucella neotomae and *Brucella microti* in wild rodents (Ko and Splitter, 2003; Khurana *et al.*, 2021).

Transmission of Brucella has been observed via horizontal or vertical routes (Khurana *et al.*, 2021). Most mammals were infected with Brucella via ingestion, mucous membranes and broken skin (Khurana *et al*., 2021). Furthermore, newborns were infected mostly due to the ingestion of contaminated colostrum or milk (Khurana *et al.*, 2021). Usually, the organism is commonly found in vaginal discharges, semen, urine, placenta, fetal contents or fluids, and milk (Khurana *et al*., 2021). The bacterium is shed by infected cows even after the full term pregnancy or abortions, mostly unnoticed by field veterinarians. The mammary gland is colonised by the organism after a systemic infection after direct entry through the teat canal from the environment (Spickler, Anna Rovid, 2018). Inhalation and conjunctivae routes of transmission have been reported in cattle. In marine mammals, the route of transmission might be by eating infected fish (Spickler, Anna Rovid, 2018).

Lipopolysaccharide (LPS) in the outer cell membrane of Brucellae is the antigen that dominates the immune response. Two forms of lipopolysaccharides created by the genus Brucella are smooth strain lipopolysaccharides (S-LPS) and non-smooth strain polysaccharides (R-LPS) (He, 2012). S-LPS consists of lipid A, distinctive fatty acids, a core region, and an O chain. The difference between S-LPS and R-LPS is that the O-chain is either absent or reduced to a few residues in the structure of R-LPS. Therefore, the specificity of the R-LPS is largely determined by the core polysaccharide in the cellular outer membrane (He, 2012).

At an early stage of life, calves can be infected without showing any clinical signs until they mature. Female animals show clinical manifestations in the form of low fertility, retention of fetal membranes, endometritis, late term abortions, giving birth to weak calves, and a reduction in milk production. Affected bulls or bull calves may develop orchitis, epididymitis and hygroma from chronic infections (Khurana *et al*., 2021).

Animal Brucellosis has been an endemic disease in Sri Lanka ever since it was first diagnosed in 1956 and has had an economic impact on the livestock industry in Sri Lanka (Priyantha, 2011; Karunanayake *et al*., 2019). For most of the rural inhabitants, dairy farming is the primary or secondary livelihood activity which consists of 1.6 million heads of cattle and buffaloes (Kothalawala *et al*., 2017). *Brucella abortus* is the common agent in bovine Brucellosis in Sri Lanka and it has been reported more common than neighbouring countries of South Asia (Bandara and Mahipala, 2002).

Vaccination of all susceptible animal hosts at risk and elimination of positive animals in endemic areas is the best way of preventing, controlling, and eradicating Brucellosis. It plays a major role in the management of the disease in animals as well as in humans. Brucella spp. strains 19, RB 51 and Rev 1 are the most commonly used very efficient vaccine strains in livestock. *B. abortus* S19 vaccine is found to be effective against *B. melitensis* as it provides cross protection. If 90% of 3-8 months old replacement heifers are vaccinated with the *Brucella* S19 vaccine, it is shown to provide excellent economic return in a Brucellosis vaccination program in bovines (Khurana *et al*., 2021). Two types of vaccination can be performed: full dose ($2.5\text{-}12x10^{10}$ colony forming units (CFU) subcutaneously or reduced dose ($3\text{-}10x10^{9}$) either through subcutaneous or conjunctival route (Dorneles *et al.*, 2015; Simpson *et al*., 2018). The objective of this study was to determine the detectable post vaccination immune response to the *Brucella abortus* S 19 live attenuated vaccine in positive and negative herds for Brucellosis in dry zone cattle. We hypothesised that vaccination with the Brucella S 19 live attenuated vaccine in cattle would show a detectable antibody response one year post vaccination.

MATERIAL AND METHODS

Epidemiological Data collection for the study

Questionnaire-based information was collected to screen the farms for reproductive disorders including abortions, infertility, the birth of weak calves and longer calving intervals in the last two years in the Vilachchiya Government Veterinary (GVS) range. Altogether, 30 units with more than 10 total heads of cattle were selected from the farm register of the GVS office at Vilachchiya.

Farm Selection for the Brucella S 19 Vaccine Experiment

Out of 30 selected farms, only 9 Farms with a previous history of abortion and 9 farms with no history of abortions were selected for the study. The rest of the 12 farms were excluded from the study.

Sample collection and processing

Animals were in 1st to 5th lactation in the 18 farms that were selected for screening by the Rose Bengal Plate (RBP) Test. With the consent of the farm owners, 10ml of blood was withdrawn into vacutainers aseptically from the jugular veins of individual animals. Altogether, one hundred and

thirty one (131) samples were collected, serum separated, and transferred to the laboratory at 4^0C. After centrifugation at 3000 rpm, the separated serum was transferred to 1.5ml sized micro centrifugation tubes and kept at -18°C until RBPT was done at the Veterinary Research Institute, Gannoruwa, Peradeniya. All RBPT positive samples were confirmed by the complement fixation test as described by Alton *et al.*, (1988) previously.

Vaccination with the *Brucella abortus* S19 vaccine

Vaccination with the *Brucella abortus* S 19 vaccine is practised only for heifer calves around 10-12 months of age in the endemic zone of Sri Lanka for bovine Brucellosis. Based on screening results for Brucellosis, three farms (n=3) with a positive history of abortions and three farms (n=3) with a negative history of abortions were selected randomly for the vaccination trial. Over 6 - month old non pregnant heifers were selected for the *Brucella abortus* S19 vaccination at 1ml each by subcutaneous route of injection (1-2 X 10^{10}cfu/ml) as recommended by Alton *et al*, 1988. Out of 28 heifers with a history of abortions, 14 were vaccinated with the *Brucella abortus* S 19 and the rest of 14 were kept as non-vaccinated controls (50%). Thirty-two (32) animals with a negative history of abortions, 16 heifers were vaccinated and rest 16 were kept as non-vaccinated controls.

Collection of samples

The blood samples were collected aseptically into a sterile vacutainer from the jugular veins of both vaccinated and unvaccinated animals at 0, 2, 4, 6, 8 and 10 weeks. All the samples were kept at room temperature for 4 hours; serums were separated after centrifugation at 3000 rpm. The serum samples were transferred to 1.5ml microcentrifuge tubes and kept at -18°C.

RESULTS

Management of information

The study was performed in the Vilahchchiya government veterinary range, a part of the recognised endemic region for bovine Brucellosis in Sri Lanka. Animals from extensively reared herds were selected for this study, and 77% of herds were found to have extensive management systems in the region (Figure 1). Abortions have been identified as a common clinical sign in extensively and semi intensively reared herds in this study. Abortion in the last trimester was shown to account for 64% of the reported cases with other reproductive disorders (Figures 2 & 3, Table 1). Moreover, the majority were found to be in close contact with wild animals (Figure 4).

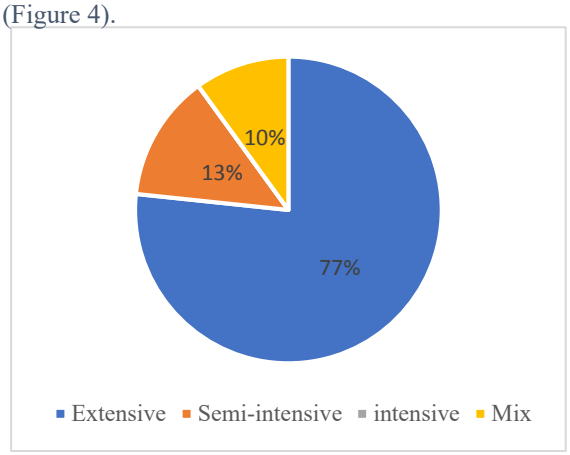

Figure: 1. Different farming system observed in Vilachchiya Veterinary range

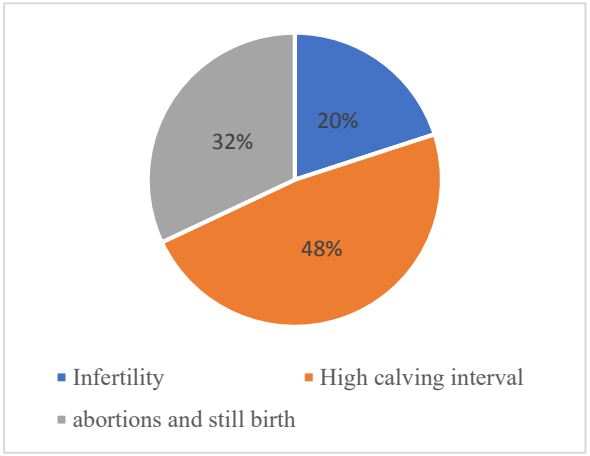

Figure 2. Common reproductive disorders observed in the study

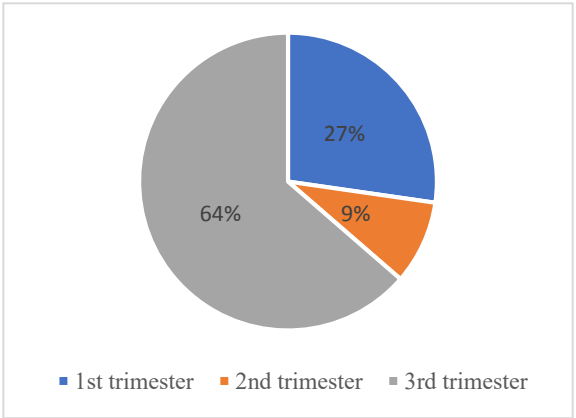

Figure 3. The time of abortions observed in the study

Table 1. Summary of vaccination and history of abortion in herds selected for the study

History of abortion and vaccination	A number of farms
History of abortions and no vaccination	11
No history of abortions and no vaccination	14
No history of abortion and with vaccination	5
Total	**30**

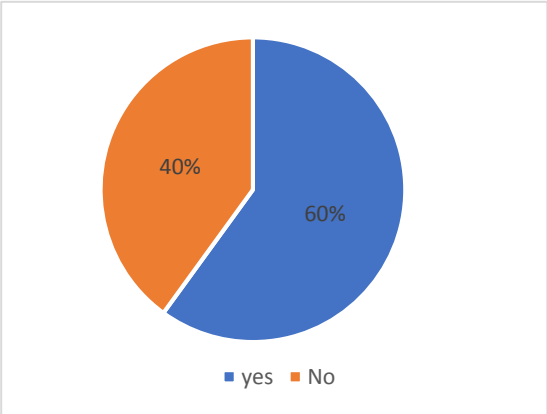

Figure 4: The cattle herds observed as contact with wild life

Immunological responses and Brucella S 19 vaccination trial

Herds with a history of abortions

The detectable immunological responses against Brucellosis were noticed by serological tests (Table 2), the Rose Bengal plate test and the complement fixation test in animals from herds with a history of abortion as detailed here; 2 weeks after the vaccination, 13 animals had between 0-1:8 of CFT titres and only one had 1:16 CFT titters (Tables 3 & 4). In the 4th week, all 14 animals had between 0 and 1:8 CFT titres. In the 6th week, 11 animals had between 0 and 1:4 CFT titres and 3 had between 1:8-1:32 CFT titres (Tables 3 & 4). All animals had 0 and1:4 CFT titres in the 8th week and no detectable CFT titres were observed and disappeared in the 10th week of post vaccination (Tables 3 & 4).

Table 2. Serum samples collection time line after the vaccination and RBPT test results.

	2nd week	4th week	6th week	8th week	10th week
Farms with a history of abortion	All positive	All positive	All negative	All negative	All negative
Farms with no history of abortions	All positive	All positive	All negative	All negative	All negative

All non-vaccinated controls were negative for Brucellosis by RBPT.

Table 3. CFT titre of individual animals from 2nd Week to 10th week (With abortion history)

Animal	Abortion History positive Farms				
	2nd week	4th week	6th week	8th week	10th week
A1	0	1:8	0	0	0
A2	1:8	1:4	1:4	0	0
A3	1:8	0	1:8	0	0
A4	0	0	1:32	0	0
B1	1:8	0	0	0	0
B2	1:4	0	0	0	0
B3	1:4	0	0	0	0
B4	1:4	0	0	0	0
B5	1:8	1:4	1:4	0	0
E1	1:16	0	0	0	0

E2	1:4	0	0	1:4	0
E3	1:4	1:8	1:16	0	0
E4	0	0	1:4	0	0
E5	0	0	1:4	0	0
Cumulative positive number	10	4	7	1	0
Cumulative negative number	4	10	7	13	14
Positive %	71.4%	28.6%	50%	7.1%	0

Interpretation: More than 1:2 CFT titter was interpreted as serologically positive for Brucellosis (Alton et al, 1988)

Table 4. CFT titre of individual animals from the 2nd week to 10th week (With negative history of abortion)

Animal	Abortion History Negative Farms				
	2nd week	4th week	6th week	8th week	10th week
C1	1:8	0	0	0	0
C2	0	0	0	0	0
C3	0	0	1:4	0	0
C4	1:4	0	1:4	0	0
C5	1:8	0	0	0	0
C6	0	0	0	0	0
F1	1:8	4	0	0	0
F3	1:16	1:16	1:8	0	0
F4	0	0	1:4	0	0
F5	1:8	1:4	1:16	0	0
F7	1:4	1:4	0	1:4	0
G1	1:8	1:8	0	0	0
G3	1:4	0	0	0	0
G4	1:8	1:4	0	0	0
G7	1:8	1:8	1:8	0	0
G9	0	0	0	0	0
Cumulative positive number	11	7	6	1	0
Cumulative negative number	5	9	10	15	16
%	68.7	43.7	37.5	6.2	0

Interpretation: More than 1:2 CFT titter was interpreted as serologically positive for Brucellosis (Alton et al, 1988)

Herds with no history of abortions

On the farm with no history of abortion, 15 animals out of 16 were found to have between 0 and 1:8 of CFT titres at the 2nd, 4th and 6th week after the vaccination. All 16 animals had between 0 and 1:4 of CFT titre at the 8th week and had no detectable antibody titres observed at the 10th week of post vaccination (Table 3 & 4). Meanwhile, no differences were observed in CFT titres or duration of detectable antibody responses in positive and negative herds for Brucellosis.

DISCUSSION

Bovine Brucellosis is an endemic disease in cattle in the dry zone of Sri Lanka (Priyantha, 2011). The Department of Animal Production and Health (DAPH) recommended vaccinating endemic cattle herds with the *Brucella abortus* S 19 live vaccine to minimize abortions and reproductive losses (Priyantha, 2011). *Brucella abortus* S 19 is a live attenuated vaccine, is used in many countries where thorough or 100% culling or slaughtering of infected animals are limited due to the lack of compensation procedures or schemes (Alton *et al*., 1988; Priyantha, 2011). The full dose of Brucella S 19 has been recommended by Shome *et al.*, (2023) as $4x10^9$cfu/ml for calves. Therefore, the alternative is to continue vaccination to reduce incidences of abortions in endemic herds or regions. In addition, post vaccination monitoring is considered a vital component of the control programme in animals (Alton *et al*., 1988). However, immunological responses to the *Brucella abortus* S 19 vaccine have not been studied in local animals and a very limited body of information has been published.

Two different doses had been identified in Brucella S 19 vaccination in cattle and buffaloes, both high (high log of colony forming unit) and reduced doses (1-3 log reduction of colony forming unit) of *Brucella abortus* S 19 vaccination are practised globally; a high dose of *Brucella abortus* S 19 may cause prolonged persistent antibody responses, which interfere with serological screening in cattle and buffaloes in the field (Simpson *et al.*, 2018; Ardiansyah *et al.*, 2019). However, both doses or inoculums (100 times or 2 logs and 1000 times or 3 logs reduction of infective dose) had been shown a more or less similar in immune responses in cattle (Shome *et al.,* 2023; Shome *et al.,* 2020). Since it is a live vaccine, *Brucella abortus* S 19 multiplies in the natural host (Alton *et al*., 1988). In the current study, immunological responses were observed for up to 10 weeks post vaccination. CFT is the gold standard test for serological diagnosis of Brucellosis in cattle and buffaloes (Alton *et al*., 1988). Two types of immunoglobulin, IgM and IgG react in screening (RBPT) and confirmatory testing (by CFT) in the serological diagnosis of Brucellosis respectively (Alton *et al*., 1988; He, 2012). In

RBPT and milk ring tests (MRT), IgM antibodies are detected in the serum of positive animals as simple platform tests while IgG antibodies are detected in CFT and ELISA with a longer laboratory protocol (Alton *et al*., 1988). Therefore, a combination of both tests is used in surveillance programs due to the high level of analytical sensitivity, specificity, practicability, and cost-effectiveness in a laboratory (Khurana *et al*., 2021). In addition, RBPT is proven to have high sensitivity and CFT has high specificity and is collectively used as a combination of diagnostic tests in global surveillance for bovine Brucellosis (Khurana *et a*l., 2021).

CFT was indicated as an IgG response to either acute infection or Brucella S 19 vaccination in ruminants (Meri *et al*, 1988). Although live vaccines create both a humeral and cell mediated immune response; the cell mediated immune response was not evaluated in the study (Barbuddhe *et al.*, 2020). In this study, a high percentage of seropositivity was observed 2 weeks post vaccination in both groups. Therefore, detectable CFT titres may be an indicator of the development of immune response in *Brucella abortus* S 19 vaccination. No significant differences were observed between those with and without a history of abortion in the study at two weeks after the vaccination. Simpson *et al*, (2018) reported similar observations previously; all animals were shown positive for RBPT in the 2nd week of post vaccination (Simpson *et al.*, 2018). Similarly, 90% of calves and adults showed high titre at 2 weeks after the vaccination. According to previous studies, in the 6th week, 50% of the animals showed over 1:4 antibody titres and 21% had over 1:8 CFT titres (Peiris, 1981). A slightly different scenario was observed in animals from abortion history negative farms; 37.5% of the animals showed over 1:4 and 18.75% had over 1:8 antibody titres in the study. Altogether, 62% of the animals showed antibody titres of less than 1: 4. All the animals were negative in the 10th week after the vaccination by CFT. The IgG titres were observed from the onset of the 2nd week post vaccination and detectable antibody titres had disappeared by the 10th week. Although this disappearance of immune indicators is faster than in other studies published, the exact reason is not known hence, another indicator needs to be found.

In Africa and Brazil, the detectable antibody titres appeared one month after post vaccination by indirect ELISA, instead of two weeks after the vaccination, as seen in our study (Poester *et al.*, 2013; Simpson *et al*., 2018). However, ELISA showed higher sensitivity than CFT in serological diagnosing of Brucellosis, ability to detect antibodies against Brucellosis is higher in ELISA than in CFT (Simpson *et al*., 2018). In cattle, detectable antibody responses were shown in 48% of the animals at one month post vaccination and 0% at 50 weeks (Simpson *et al.*, 2018). However, the exact reason for the fast disappearance of immune responses in this study is not known. Multiple factors, such as the dose, standard practices of vaccination, number of colony forming units of bacteria at the inoculation, and breed characteristics are assumed to contribute to the persistent and detectable antibody responses in cattle. Furthermore, the viable bacterial count of organisms can be reduced in dissolved vaccine; the importance of the cold chain is very important during successful vaccination in cattle and buffaloes (Alton *et al*., 1988). However, extensive immunological studies are required for better clarification.

In the quantitative analysis of CFT titres in abortion history-positive farms, 71.42% of the animals showed over 1:4 CFT levels by 2 weeks after the vaccination while the rest 28.57% were negative for CFT, irrespective of the fact that all of them were positive by RBPT. Although antibody titres against infection and vaccination are challenging in CFT, more than 1:2 titres were serologically positive for bovine Brucellosis (Alton, *et al*., 1988). In contrast, additional booster vaccinations one year after the first vaccination showed a greater immune response than a single vaccination in cattle (Dorneles *et al*., 2015).

CONCLUSION

Brucellosis is an important endemic disease among extensively rared cattle in the dry zone of Sri Lanka. No persistent and detectable antibody responses were found 10 weeks post vaccination of female calves in cattle herds in the endemic zone. It implies that a positive result for CFT indicates a sign of an infection of Brucellosis rather than a post vaccination effect in the dry zone. Although detectable antibody responses had been observed previously at 28 weeks of post vaccination in cattle, rapidly diminishing antibody responses were observed in our study, in contrary to the literature. Since CFT has not been considered a sensitive indicator of immunological response, further studies are recommended with highly sensitive tests such as ELISA in the future.

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