ejpmr, 2023, 10(8), 280-288



# EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article ISSN 2394-3211 EJPMR

# HAEMATO-BIOCHEMICAL CHANGES AND PREVALENCE OF ZOONOTIC BACTERIA ISOLATED FROM MARKETED INDIGENOUS CHICKENS IN KIAMBU COUNTY, KENYA

# P. Wamboi\*<sup>1,3</sup>, J. Nguhiu-Mwangi<sup>2</sup>, P. G. Mbuthia<sup>1</sup>, R. M. Waruiru<sup>1</sup> and L. C. Bebora<sup>1</sup>

<sup>1</sup>Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi-Nairobi, Kenya.

<sup>2</sup>Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi-Nairobi, Kenya.

<sup>3</sup>Regional Veterinary Investigations Laboratories Mariakani, Directorate of Veterinary Services, P.O. Box 204-80113, Mariakani-Kilifi, Kenya.

### \*Corresponding Author: P. Wamboi

Regional Veterinary Investigations Laboratories Mariakani, Directorate of Veterinary Services, P.O. Box 204-80113, Mariakani-Kilifi, Kenya.

Article Received on 27/06/2023

#### Article Revised on 30/06/2023

Article Accepted on 01/07/2023

## ABSTRACT

This study investigated the safety status of indigenous chicken sold in markets of Kiambu County Kenya, mainly for human consumption and the likely associated haemato-biochemical changes. Thirty chickens were purchased from the county markets, the body condition assessed and manifesting clinical signs recorded. Swabs for bacteriology using conventional methods, were aseptically taken from the cloaca, oropharynx, liver and spleen. Forty-seven percent (47%; 14/30) of the chicken were in a poor state, 43% (13/30) in fair, and only 10% (3/30) exhibited good body condition. Ten bacteria genera were identified from 244 isolates in the four organs. The cloaca had (102/244; 41.8%), the oropharynx (113/244; 46.3%), the spleen (22/244; 9.0%) and liver (7/244; 2.9%). Escherichia coli were the most prevalent (93.3%) while Serratia and Pseudomonas were the least prevalent (3.3%) each. Relative to normal values the mean basophil and band cells were significantly high (p=0.05; p=0.001) in chicken with bacteria in the liver. Both mean serum ALT levels (p=0.0005) and mean Band cells value (p=0.0014) were significantly high in chicken with bacteria in the oropharynx. This study demonstrated that the marketed chickens were carriers of known pathogenic and zoonotic bacteria, example; Escherichia coli, Streptococcus agalactiae, Listeria monocytogenes, Campylobacter coli and jejuni. Presence of bacteria in the liver and spleen indicated bacteremia which may contribute to alteration of haemato-biochemical parameters of subclinically infected chickens. These findings are expected to facilitate and encourage hygienic handling of poultry meat to avoid cross-infection and co-infection, and use of clinical-pathology to measure poultry health status for enhanced disease diagnoses.

KEYWORDS: Bacteria isolates, indigenous chicken, zoonoses, market cross-infection, haemato-biochemical.

# INTRODUCTION

Poultry, primarily chickens, are the most extensively reared livestock type worldwide and the most abundant species (Permin and Hansen, 1998; Kingori *et al.*, 2010). Their products have been reported as one of the most vital and preferred source of animal protein for man worldwide (Permin and Hansen, 1998). The global population of poultry is projected at approximately 16.2 billion, of which 71.6% is in developing countries (Nduthu, 2015). More than 80% of human population in East Africa lives in rural areas, with more than 75% of these keeping indigenous chickens (Kingori *et al.*, 2010; Nduthu, 2015; Ahmed, 2018). Kenya has an estimated population of over 37.3 million domesticated birds. Among these, 31.578 million (84.6%) are indigenous chickens, 3.1 million (8.3%) are layers and while 2.1

L

million (5.6%) are broilers. The remaining 0.522 million (1.4%) constitute other poultry species (MOLFD, 2007). Despite the large proportion of chickens kept in rural areas, there is scarce data published on research findings in rural poultry health and the likelihood of its contribution to zoonosis.

According to Ahmed (2018), the major challenges encountered in poultry production include disease, predation, feed shortage and scarcity of information on appropriate indigenous poultry health practices. Coinfections including concurrent diseases are a common finding in poultry production. This is often subclinical bacterial infections accompanied by ecto- and endoparasite infestations.

Some bacterial diseases of chicken are of importance, resulting in high mortality under rural conditions (Permin and Pedersen, 2002). These include diseases caused by *Escherichia coli*, which affect all ages, but especially chicks; non-typhoid salmonellae, which affect all ages, but mainly chicks (*Salmonella pullorum* in chicks less than 3 weeks of age and *Salmonella gallinarum* in growers and adults). Others include *Pasteurella multocida* in growers and adults; *Avibacterium paragallinarum* causing Infectious coryza in growers as well as adults; *Clostridium perfringens* in all ages, but mainly in growers; *Mycobacterium avium* in adults; *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in all ages.

Methods of indigenous chicken production, mostly backyard or free-range systems, entail low biosecurity measures. Hence, a high risk of infectious diseases including zoonoses (Conan *et al.*, 2012). Examples of infectious bacterial diseases of poultry with zoonosis importance include: campylobacteriosis, listeriosis, mycobacteriosis, collibacillosis, salmonellosis and staphylococcosis (Whitehead and Robert, 2014; Nga *et al.*, 2019). Campylobacteriosis is a top foodborne zoonosis globally, frequently linked with handling and consumption of poultry meat. Various studies have shown that *Campylobacter* organisms cause a significant burden of human disease in low to middle-income countries (Carron *et al.*, 2018).

Most of *Echerichia coli* strains occur as commensals in gastro-intestinal tract of animals and humans, however, some that have shown pathogenicity to varying degrees. The most important is enterohaemorrhagic *E. coli* (EHEC), particularly the O157:H7 serotype which causes hemorrhagic diarrhea and kidney damage in humans. Septicemia due to *E. coli* is also a severe, potentially deadly disease of fowls and humans (WHO, 2018; Wigley *et al.*, 2013; Kaper *et al.*, 2004; Ratnam *et al.*, 1988).

Salmonellosis is another problem globally for both animals and humans. Most of *Salmonella enterica* serotypes infect many animal species causing disease and diarrhoea in humans. The most significant foodborne *Salmonella* serotypes are *Salmonella* Typhimurium and *Salmonella* Enteritidis in terms of number of cases and severity of infection (Wigley *et al.*, 2013). This study investigated the safety status of indigenous chicken sold in selected markets of Kiambu County Kenya, mainly for human consumption.

Haematological and biochemical parameters are good indicators of the health assessment for both animals and humans and yet they are rarely used. Sub-clinical bacterial infections may be contributing towards morbidity losses experienced by poultry farmers. It is, therefore, of interest to monitor changes in haematobiochemical parameters of marketed chickens to establish their respective levels as some of these

L

parameter-changes could be associated with bacterial infections. Limited studies have been done on assessment of clinico-pathological parameters in poultry disease diagnoses (Permin and Pedersen, 2002; Wamboi *et al.*, 2020).

The purpose of this study was to determine safety and health statuses of marketed indigenous chickens. Determine the prevalence of pathogenic and zoonotic bacteria prevalence in sub-clinically infected, marketed indigenous chicken, and the probable associated haemato-biochemical changes. Results of this study are projected to add towards and encourage use of clinicopathological parameter analysis in assessing poultry health status. Second. creation of awareness to: 1) farmers on the risk posed by disposing off sick birds by selling them to ignorant consumers in the markets, 2) purchasers of respective chickens for consumption to ensure the meat is properly cooked prior to consumption, 3) handlers of poultry meat to observe strict hygiene, where possible to wear protective gear so as to minimize contamination and infections, and 4) chicken farmers purchasing for restocking to ensure the new stock is isolated/quarantined and treated appropriately before mixing with other farm birds to avoid introduction and spread of the bacterial diseases.

# MATERIALS AND METHODS

#### Study area

This study was conducted in three chicken markets (Wanginge, Uthiru, Gitaru) located in Kabete and Kikuyu Sub-Counties of Kiambu County, Kenya; they were taken as representatives of the County due to the large numbers of chicken sold in these markets. The physiographic and natural land conditions of the study area are as described in Kiambu Agriculture, Livestock & Fisheries 2018-2021 Strategic Plan (Anonymous, 2018). Kiambu County was selected for the study because the chicken markets in Kabete and Kikuyu sub-counties are conveniently in close proximity to the Faculty of Veterinary Medicine, University of Nairobi that was the analysis site. Furthermore, the major poultry markets in Kenya are located within Kiambu County (Kothari, 2004).

# Study design

A purposive sample taking design was used for the study. This was based on convenient sampling where chickens were obtained from purposively selected market centres. The study was cross-sectional. Thirty indigenous chickens were randomly selected and purchased from the three chicken markets. The sample size of study chickens was calculated based on the formula by Martin *et al* (1987) and Kothari (2004). Market distribution of the birds was: 12 (Uthiru), 12 (Waginge) and 6 (Gitaru). Information on the possible origins of the marketed chickens was captured through administration of a simple questionnaire to the chicken traders. All the chickens were transported alive and untethered in cages, immediately after purchase, to the

I

Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi (UoN) where they were physically examined before and after being killed humanely; then samples were aseptically taken for bacterial isolation and identification.

#### Ethical approval

The Faculty of Veterinary Medicine Biosafety Animal use and Ethics Committee (BAUEC) approved this study; approval number is REF: FVMBAUEC/2018/177.

#### Determination of body condition of chickens

The body condition was as reported by Wamboi et al. (2020). The method used for scoring was as described by Gregory and Robins (1998); the scoring scale ranged from 0 to 3 based on prominence of the keel bone.

#### Sample collection, handling and transportation

After external physical examination and killing of the chicken, cloacal, oropharyngeal, hepatic and splenic swabs were taken for bacterial isolation and identification. Sampling was done in strict asepsis using sterile bacteriology swab under Bunsen burner flame. The swabs were placed in Stuarts transport media, labelled and immediately transported to the bacteriology laboratory for processing (McVey *et al.*, 2013).

#### Bacterial isolation and characterization

Primary culture was done on Sheep blood agar (SBA), MacConkey (MAC) agar, Cystine Tellurite blood agar (CTBA), incubated aerobically for 24 hours at  $37^{\circ}$  C. Culture was also done in Campylobacter Agar Base (Karmali) mixed with Campylobacter selective supplement (Karmali), SR0167E, incubated under microaerophilic condition at  $37^{\circ}$ C for 48 hours (Oxoid, Kenya manufactured Media) for isolation of *Campylobacter spp*.

Sub-culture was done following primary culture to obtain pure colonies of the isolated bacteria. From MAC, all non-lactose fermenters were sub-cultured onto Salmonella Shigella Agar (SSA); all lactose fermenters were sub-cultured onto Eosin Methylene Blue (EMB) Agar. From SBA all the colonies were sub-cultured onto nutrient agar for biochemical test analysis (Markey et al., 2013; Pohjola, 2017). The isolated E. coli were further cultured on Sorbitol MacConkey Agar and characterization done to specifically check for presence of the serotype O157:H7; Enterohaemorrhagic E. coli (EHEC).

Identification of bacterial pathogens was done centered on growth pattern and colony features on primary, selective and differential media. Additionally, Gram stain, IMViC, Catalase, Oxidase, Urease and motility tests were done according to standard test procedures for identification of isolates (Steubing, 1993; Mathan *et al.*, 2013). *Streptococcus* organisms were confirmed through growth on Sodium azide crystal violet blood agar (SACVBA), while respective species differentiation was done through Christie, Atkins and Muench-Peterson (CAMP) test, as described by Bae and Bottone (1980).

*Listeria* spp. was isolated using the selective medium Cysteine tellurite blood agar (CTBA) after refrigeration at  $4^{\circ}$ C overnight (Bae and Bottone, 1980; Njagi *et al.*, 2004). Members of genus *Campylobacter* were detected using polymerase chain reaction (PCR) technique after culture of the organism (Miller *et al.*, 2006). Other bacteria were characterized through biochemical tests after culture.

#### Analysis of haemato-biochemical parameters

Haemato-biochemical parameters analysis was as reported by Wamboi et al. (2020). Haematological parameters were analysed as described by Coles (1976) and Dacie and Lewis (1991), while biochemical parameters were analysed as described by Doumas (1975), Bergmeyer et al. (1978) and Sakas (2002).

#### Data management and analysis

Data was entered into Microsoft Office Excel 2016 spreadsheets. It was cleaned then verified as correct entries from the data collection book. The data was imported into Statistical Package for the Social Sciences SPSS version 22 (Corp, 2013). Percentages of occurrences of isolated bacteria were calculated out of the total bacterial isolates. A critical probability of 0.05 was adopted throughout as a cut-off point for statistical significance.

# RESULTS

#### Origin of the marketed indigenous chicken

The chicken being sold at the various markets of Kiambu County from where the study birds were purchased, had been supplied from the various Sub-Counties of Kiambu County that is; Limuru, Ndeiya and Gatundu and others were supplied from Bomet County.

# Body condition and physical examination findings of the study chickens

On physical examination, 47% of the chickens were found to be in poor body condition, 43% were fair and only 10% were in good body condition (Wamboi *et al.*, 2020). Table 1 below presents the various clinical manifestations and their corresponding body conditions.

Body	Diarrhoea		<b>Respiratory distress</b>		Wounds		Deplumage	
condition	n=30	%	n=30	%	n=30	%	n=30	%
Poor	8	26.7	12	40	16	53.3	1	3.3
Fair	5	16.7	12	40	12	40	1	3.3
Good	0	0	0	0	0	0	0	0
Total affected	13	43.3	24	80	28	93.3	2	6.7

 Table 1: Various clinical manifestations and their corresponding body conditions as observed on the study chickens.

#### Bacteria isolated from various chicken organs

Total number of isolates were 244; from oropharynx 46.3% (113/244), cloaca 41.8% (102/244), spleen 9.0% (22/244), and liver 2.9% (7/244). Ten bacterial genera

were identified, of these, six were identified to species level. Their respective prevalence was as illustrated in Table 2.

Table 2 : Prevalence of bacteria isolate types from various organs and sites in the study chick	ens.
---	------

Bacterial isolate types	Organ/site from which bacteria were isolated							
Number	Cloaca		Oropharynx		Spleen		Liver	
(n=30) for each organ/tissue	Number	%	Number	%	Number	%	Number	%
Escherichia coli	28	93.3	22	73.3	4	13.3	0	0
Staphylococcus aureus	6	20	16	53.3	4	13.3	2	6.7
Other Staphylococcus	10	33.3	20	66.7	6	20	2	6.7
Streptococcus agalactiae	0	0	1	3.3	0	0	0	0
Other Streptococcus	11	36.7	12	40	2	6.7	0	0
Proteus species	4	13.3	3	10	1	3.3	0	0
Bacillus species	13	43.3	7	23.3	0	0	0	0
Listeria monocytogenes	0	0	4	13.3	0	0	0	0
Other Listeria species	24	80	22	73.3	4	13.3	2	6.7
Serratia marcescens	0	0	1	3.3	0	0	0	0
Micrococcus species	0	0	3	10	0	0	0	0
Pseudomonas species	0	0	1	3.3	1	3.3	1	3.3
Campylobacter jejuni	4	13.3	1	3.3	0	0	0	0
Campylobacter coli	2	6.7	0	0	0	0	0	0
Total number of isolates (244)	102	41.8	113	46.3	22	9.0	7	2.9

*Key: n* - chicken sample size and (%) - Percentage.

#### Haemato-biochemical changes

The mean haematological and biochemical parameters that showed variation from normal documented range values were as reported by Wamboi et al. (2020). Chickens with bacterial infection in the liver had significantly (p = 0.0001) higher mean Band cells value (10.75%) than those without (1.88%). Basophil value was also significantly (p = 0.05) higher (0.75%) in those with bacterial infection relative to the non-infected (0.12%) Table 3.

Table 3 : Effect of presence of bacteria in the liver of study chicken on mean haematological parameters.

Heemeteleau neverators	Liver bact	eria isolates			
Haematology parameters	Present	Absent	t-value	p-value	
Haematocrit (%)	34.08 35.61		-0.55	0.5854	
Erythrocyte count (× $104\mu$ l)	240.38	229.79	0.34	0.7360	
Leucocyte count (× $103\mu$ l)	57.88	45.72	0.74	0.4660	
Platelet count	42.75	35.39	0.48	0.6361	
Lymphocyte (%)	36	31.58	0.63	0.5341	
Monocyte %	14.75	15.93	-0.27	0.7920	
Heterophil %	30.5	41.62	-1.35	0.1864	
Eosinophil %	7.25	8.58	-0.32	0.7512	
Band cells %	10.75	1.88	4.44	0.0001*	
Basophil %	0.75	0.12	2.04	0.05*	

I

*Key:* \*Significant difference at p < 0.05, (%) percentage, (µl) microliter.

Presence of bacteria in the oropharynx showed a significant increase in the serum ALT mean levels

L

(p=0.0005) and also a significant increase in the mean value of Band cells (p=0.0014). Presence or absence of

WWW PI	pmr.com

bacteria in the spleen and cloaca exhibited no significance difference on the tested haematological and biochemical parameters when their means were compared statistically. Presence or absence of bacteria in the liver had no significant difference on the biochemical parameters tested.

## DISCUSSION

The study revealed that local, village indigenous chickens marketed in Kiambu County, Kenya, harboured both pathogenic bacteria and non-pathogenic bacteria some of which have zoonosis implication, including members of genera *Escherichia*, *Staphylococcus*, *Streptococcus*, *Listeria*, *Pseudomonas*, *Campylobacter*, *Proteus* and *Bacillus*. This is of great significance considering most of these chicken end up being consumed within the vicinity of Nairobi City County and its metropolitan residents.

It is notable that Escherichia coli organisms were the most isolated from the various organs and sites of the study chickens, which could be of public health concern. Despite most strains of this bacteria being normal flora within gastro-intestinal tract (WHO, 2018), some have been found to be pathogenic causing various forms of coliform infections in chickens and humans (Kaper et al., 2004; WHO, 2018), that may result in various clinical symptoms ranging from mild diarrhea to serious hemorrhagic diarrhea and septicemia. Since village chickens are normally raised free-range, these bacteria are easily spread through faecal discharge within the homestead environment and could end up contaminating foods such as vegetables, some of which are eaten without cooking. This puts the village population at risk of contracting infections. Although E. coli serotype 0157: H7 has been documented as a cause of hemorrhagic diarrhoea and kidney damage in humans (Ratnam et al., 1988; Kaper et al., 2004; WHO, 2018), it was not isolated in this study. However, Bebora et al. (1993) recovered it from a case of septicaemia in chicks. This suggests that chickens could carry this enterohemorrhagic serotype, thus a potential source of human infection. This could be a possibility from marketed indigenous chicken that are kept, transported, sold and handled with minimal or no biosecurity considerations.

The high prevalence of *Staphylococcus* among the bacteria isolated from the various organs and sites of the study chickens is an important consideration for consumers of indigenous poultry meat and products. *Staphylococcus aureus* is common in birds and commonly occurs in bones, tendon sheaths and leg joints (Cheville *et al.*, 1988). Devriese et al. (1983) reported presence of *Staphylococcus aureus* is a key pathogen in humans that causes various clinical infections, being a primary cause of bacteremia and infective endocarditis. It is also a cause of osteoarticular, skin, soft tissue, pleuropulmonary, and device-related infections (referring to the host response to one or more microbial pathogen

L

on or in an indwelling device/implant) in humans (Tong et al., 2015).

The isolation of Streptococcus organisms from oropharynx and cloaca of the study chickens can be explained by the previous report that this bacteria species is a normal intestinal flora of many avian species, including wild birds (Brittingham, et al., 1988). The association of oropharynx and cloaca with the alimentary tract helps to confirm the reason for isolation of Streptococcus. Streptococcal infections in poultry can be localized or systemic resulting in septicemia; they can be acute or chronic. While septicemia may manifest as endocarditis and/or lameness in affected birds (Devriese et al., 2002; Chadfield et al., 2004), Streptococcus, especially Streptococcus pyogenes, or group A Streptococcus (GAS), causes mild infections such as pharyngitis and impetigo and serious infections such as necrotizing fasciitis and streptococcal toxic shock syndrome in humans (Walker et al., 2014).

Isolation of Listeria monocytogenes and other Listeria species from the study chickens that did not manifest any symptoms of disease was consistent with the findings of other investigators (Marsden, 1994; Zander et al., 1997; Njagi et al., 2004). This genus of bacteria can have strains that are potentially pathogenic. While other Listeria spp. were isolated from all the four organs sampled, Listeria monocytogenes was obtained from oropharynx, and only in four of the 30 study chickens, which was similar to the findings of previous studies done in Kenva (Njagi 2003; Njagi et al., 2004). Previously, Njagi (2003)isolated Listeria monocytogenes from only 2 out of 40 indigenous chickens obtained from the market. However, in that previous study, Njagi (2003) did not isolate Listeria species from the cloaca, contrary to the current study in which Listeria were isolated from the cloaca of the study chickens at 80%. In humans, L. monocytogenes is the most important Listeria spp, causing pregnancy losses in healthy women, and septicaemia or central nervous disease in immuno-suppressed, system (CNS) debilitated, newborn or elderly persons (Saleh et al., 2012).

*Pseudomonas*, though notably isolated at low prevalence from the oropharynx, spleen and liver of the study chickens, can be a serious poultry pathogen and a zoonotic bacterium especially *Pseudomonas aeruginosa*, as reported by Elsayed et al. (2016). Kebede (2010), in his study, established that *Pseudomonas* infection was associated with increased mortality in recently hatched chicks and embryos. This renders *Pseudomonas* infection a serious economic challenge in poultry farms. In chicken, *Pseudomonas* pathogenicity is associated with respiratory infection, keratoconjuctivitis, sinusitis and septicaemia and soared embryonic death rates in hatcheries (Saif *et al.*, 2003; Hai-ping, 2009). In humans, it is considered an opportunistic organism majorly associated with nosocomial infections that are difficult to

I

treat. It is also associated with a broad range of diseases such as urinary infections, burns, respiratory infections, and septicemia. It is the chief cause of ventilationassociated pneumonia in human intensive care units (Fazeli *et al.*, 2012).

Campylobacter species, especially Campylobacter jejuni, have been reported as common isolates from faeces (Hughes and Rees, 1997) and this may explain isolation of *Campylobacter coli* and *Campylobacter jejuni* mainly from cloaca and oropharynx of the study birds. It has also been established that chickens are carriers of Campylobacter (Osano and Arimi, 1999), thus explaining the possibilities of isolating them from indigenous chicken. Isolation of Campylobacter is of major importance in chickens sold for consumption in the market owing to its zoonotic potential. Campylobacter coli and C. jejuni are among the most common causes of human gastroenteritis in the world. Although the food poisoning caused by the organisms can be severely debilitating, it is rarely life-threatening, but it has been linked with subsequent development of Guillain-Barré syndrome, which usually develops two to three weeks after the initial illness (Carron et al., 2018; Fujimoto and Amako, 1990).

The high prevalence of bacteria isolates in this study, with some being pathogenic, may be attributed to nonobservance of biosecurity and disease preventative measures during the rearing of the chickens at the farmlevel as well the practice of mixed farming where the chickens are in close proximity with other domestic animals that could be shedding both pathogenic and nonpathogenic bacteria. The chickens may pick bacteria environment. from the contaminated including scavenging on manure and beddings of other domestic animals (Sanaa et al., 1993) and probably mixing with chickens from other neighboring homes. At the market, cross-infections from chicken to chicken is inevitable, as the chickens share cages/places prior to being sold.

Another study, investigating the same cohort of chickens for parasite carriage and hemato-biochemical changes (Wambui et al., 2020) made observations that may support presence and occurrence levels of the bacteria in the study chickens. The study demonstrated that the birds were infested with endoparasites (nematodes - Heterakis gallinarum, Sublura brumpti and Allodapa suctoria; cestodes - Raillietina echinobothrida, Hymenolepi,s and protozoa - Eimeria species), ectoparasites (lice -Menacanthus stramineus, Liperus caponis, Goniocotes gallinae, and Echinophaga gallinaceae (stick-tight flea), and hemoparasites (Leucocytozoon spp., Plasmodium spp. and Agyeptiniella spp, Haemoproteus spp), which tend to cause stress to the birds through competition for nutrients, blood sucking and irritation (Magwisha et al., 2002; Bala et al., 2011; Tamiru et al., 2014; Angyiereyiri et al., 2015; Taylor et al., 2015; Zeryehun and Yohannes 2015; Berhe et al 2019). Stress causes immunesuppression (Dhabhar, 2009), thus enabling bacteria to

L

multiply and establish themselves. Observation of the various clinical signs when the birds were examined at the market place (poor holding conditions, signs of diarrhea, respiratory distress) is indication that the birds had underlying diseases; some deliberately being disposed by the farmer for economic reasons selfishly even after they noticed that the birds were sick, which would take advantage of unsuspecting buyers in the market. The significant changes depicted in the tested haemato-biochemical parameters are also an indication of disease in the chickens. However, there is a need for controlled studies to expound on the association of blood haemato-biochemical parameters and bacterial infection in domestic chickens.

Bacterial isolation from the spleens and livers is an indicator of the chickens being sick (bacteraemia/septicaemia); meaning that these birds are sources of virulent bacteria, some of which can cause disease to humans, on consumption. Mohammed (2014), in his study, documented isolation of E. coli from liver of naturally infected chickens. This was also shown in an experimental study done on turkeys by Arp (1982), while Bebora et al. (1979) isolated Salmonella organisms from livers of naturally infected chickens. This, therefore, is an alert that some of the chicken sold in the markets may be sick and owners are disposing them to avert loss through death of the chicken. Buyers purchasing chickens for consumption should therefore ensure proper inspection and cooking prior to use of their meat for food. Chicken(s) purchased for restocking should, be isolated/quarantined and treated appropriately before mixing with the other birds, in order to avoid introduction and spread of the bacteria and respective diseases to existing stock in the farm. In addition, those handling and/or slaughtering the birds need to be made aware that they could acquire infections from the birds and be encouraged to wear protective clothing while at work.

#### CONCLUSION

Some of the indigenous chickens sold in markets are clinically sick, as indicated by isolation of bacteria from the liver and spleen (bacteraemia or septicaemia) during the study. The chickens harbor bacteria of economic and public health importance.

- Some of the bacteria isolated from these indigenous chickens and which are of public health importance include: *Escherichia coli, Campylobacter coli, Campylobacter jejuni, Listeria monocytogenes,* other *Listeria* spp., *Staphylococcus aureus,* other *Staphylococcus* spp. and *Serratia marcescens*
- The marketed birds were stressed, as a result of coinfections with parasite burden coupled with roughhandling during transportation and holding time in the market, as indicated by presence of wounds on their bodies.

#### RECOMMENDATIONS

- Creation of awareness to chicken traders and consumers of the possible infections that can arise from indigenous chickens being sold in the markets.
- Training of chicken traders as well as small-scale farmers on available control strategies against bacteria, especially those of zoonotic and economic importance.
- Creating awareness on humane handling of the birds as they are taken to the market. Apart from harming the birds, stress causes immune-suppression, thus allowing higher multiplication of bacteria resident in the birds.
- Development and enforcement of a policy addressing strict poultry meat inspection as well as proper cooking of chicken meat or products. This is to ensure that marketed chickens and/or their products are safe for human consumption.
- Regular disease control including deworming and practice of biosecurity procedures in rearing of indigenous chicken to promote health and reduce incidences of co-infections.
- There is a need for controlled studies to expound on the association of blood haemato-biochemical parameters and bacterial infection in domestic chickens.

#### **CONFLICT OF INTERESTS**

All authors declare that they have no conflict of interest.

#### ACKNOWLEDGEMENTS

We acknowledge the enormous support and contribution from; the co-authors and supervisors, the laboratory technicians, and the University of Nairobi (Department of Veterinary Pathology, Microbiology and Parasitology and Department of Clinical Studies) in making this study a success.

#### FUNDING

This study was financially supported by Cunningham scholarship through Borlaug Higher Education for Agricultural Research and Development (BHEARD).

#### REFERENCES

- Ahmed M. Major constraints and health management of village poultry production in Ethiopia: review school of veterinary medicine, Jimma University, Jimma, Ethiopia. Journal of Research Studies in Microbiology and Biotechnology, 2018; 4(1): 1-0.
- 2. Angyiereyiri ED, Sackey I, Bonu-Ire MS. Survey on arthropod ectoparasites on goats and domestic fowls in Vunania, Navrongo, Ghana, 2015; 9: 3371-3377.
- 3. Anonymous: Kiambu Agriculture, Livestock & Fisheries 2013-2017 Strategic Plan. 2014. (http://www.kiambu.go.ke/about/position-size
- 4. Arp LH. Pathology of spleen and liver in turkeys inoculated with Escherichia coli. Avian Pathology, 1982 Jan 1; 11(2): 263-79.

L

- 5. Aujla RS and Patel R. Creatine Phosphokinase. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Bookshelf ID: NBK546624PMID: 3153623;2021.
- 6. Bae BH, Bottone EJ. Modified Christie–Atkins– Munch-Petersen (CAMP) test for direct identification of hemolytic and nonhemolytic group B streptococci on primary plating. Canadian journal of microbiology, 1980 Apr 1; 26(4): 539-42.
- Bala AY, Anka SA, Waziri A, Shehu H. Preliminary survey of ectoparasites infesting chickens (Gallus domesticus) in four areas of Sokoto Metropolis. Nigerian Journal of Basic and Applied Sciences, 2011; 19(2).
- 8. Bebora LC, Oundo JO, Khamala J, Saidi S, Sang WK, Yamamoto H and Mukundi PW. Some *E. coli* strains causing septicaemia in chicks in Kenya. The Kenyan Veterinarian, 1993; **17**: 1-2.
- 9. Bebora LC, Waiyaki PG, Kaminjolo JS. A Bacteriological And Serological Survey Of Avian Salmonellosis In Four Farms And A Slaughterhouse. Kenya Veterinarian, 1979; 3(2): 36-38.
- 10. Bergmeyer HU, Scheibe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Am Assoc Clin Chem, 1978; 1: 58–73.
- Berhe M, Mekibib B, Bsrat A, Atsbaha G. Gastrointestinal helminth parasites of chicken under different management system in Mekelle town, Tigray region, Ethiopia. Journal of Veterinary Medicine, 2019; 7. Article ID 1307582. DOI:10.1155/2019/1307582.
- Brittingham MC, Temple SA, Duncan RM. A survey of the prevalence of selected bacteria in wild birds. Journal of Wildlife Diseases, 1988 Apr; 24(2): 299-307.
- Carron M, Chang YM, Momanyi K, Akoko J, Kiiru J, Bettridge J, Chaloner G, Rushton J, O'Brien S, Williams N, Fevre EM. Campylobacter, a zoonotic pathogen of global importance: Prevalence and risk factors in the fast-evolving chicken meat system of Nairobi, Kenya. PLoS neglected tropical diseases, 2018 Aug 13; 12(8): e0006658.
- 14. Chadfield MS, Christensen JP, Christensen H, Bisgaard M. Characterization of streptococci and enterococci associated with septicaemia in broiler parents with a high prevalence of endocarditis. Avian Pathology, 2004 Dec 1; 33(6): 610-617.
- 15. Cheville NF, Tappe J, Ackermann M, Jensen A. Acute fibrinopurulent blepharitis and conjunctivitis associated with Staphylococcus hyicus, Escherichia coli, and Streptococcus sp. in chickens and turkeys. Veterinary Pathology, 1988 Sep; 25(5): 369-375.
- 16. Coles EH. Veterinary clinical pathology. 4th ed. Philadelphia: W.B. Saunders, 1986.
- 17. Conan A, Goutard FL, Sorn S, Vong S. Biosecurity measures for backyard poultry in developing countries: a systematic review. BMC veterinary research, 2012; 8(1): 240 Statistics IS.
- 18. Dacie JV, LewisSM. Practical textbook of

I

haematology. 7th ed. Edinburgh: Church Livingstone, 1991; 37–85.

- 19. Devriese L, Cauwerts K, Hermans K, Wood AM. Enterococcus cecorum septicemia as a cause of bone and joint lesions resulting in lameness in broiler chickens. Vlaams Diergeneeskundig Tijdschrift, 2002; 71(3): 219-221.
- 20. Devriese LA, Poutrel B, Kilpper-Bälz R, Schleifer KH. Staphylococcus gallinarum and Staphylococcus caprae, two new species from animals. International Journal of Systematic and Evolutionary Microbiology, 1983 Jul; 33(3): 480-486.
- Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation, 2009; 16(5): 300-317.
- 22. Doumas BT. Standards for total serum protein assay. Clin Chem, 1975; 21: 1159–1166.
- 23. Elsayed MS, Ammar AM. Al shehri ZS, Abd-El Rahman H and Abd-El Rahman NA. Virulence Repertoire of Pseudomonas aeruginosa from some Poultry Farms with Detection of Resistance to Various Antimicrobials and Plant Extracts. Cellular and Molecular Biology, 2016; 62(1): 124.
- 24. Fazzeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens, 2012; 17(4): 332-337
- 25. Gregory NG, Robins JK. A body condition scoring system for layer hens. New Zealand Journal of Agricultural Research, 1998 Dec 1; 41(4): 555-559.
- Hai-ping HE. Isolation and identify of Pseudomonas aeruginosa in chicken dead-embryos. Chinese Qinghai J Anim Vet Sci, 2009; 3: 25-27.
- Hughes RA, Rees JH. Clinical and epidemiologic features of Guillain-Barré syndrome. Journal of Infectious Diseases, 1997 Dec 1; 176(Supplement\_2): S92-S98.
- IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Google Search, 2013.
- 29. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nature reviews microbiology, 2004 Feb; 2(2): 123-140.
- Kebede F. Pseudomonas infection in chickens. J. Vet. Med. Anim. Health, 2010 Nov; 2(4): 55-58.
- Kingori AM, Wachira AM, Tuitoek JK. Indigenous chicken production in Kenya: a review. International Journal of Poultry Science, 2010; 9(4): 309-316.
- 32. Kothari CR. Research methodology: Methods and techniques. New Age International, 2004.
- 33. Magwisha HB, Kassuku AA, Kyvsgaard NC, Permin A. A comparison of the prevalence and burdens of helminth infections in growers and adult free-range chickens. Tropical Animal Health and Production, 2002 May; 34: 205-214.
- Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical veterinary microbiology ebook. Elsevier Health Sciences, 2013 Nov 30.
- 35. Marsden JL. Industry perspective on Listeria

L

monocytogenes in foods: Raw meat and poultry. Dairy, food and environmental sanitation: a publication of the International Association of Milk, Food and Environmental Sanitarians (USA), 1994; 14: 83-86.

- 36. Martin SW, Meek AH, Willeberg P. Veterinary epidemiology Principles and Methods Iowa State University Press. Ames First Edition, 1987; 265.
- Mathan S, Subramanian V, Nagamony S, Ganapathy K. Isolation of endophytic fungi from marine algae and its bioactivity. Int. J. Res. Pharm. Sci, 2013; 4(1): 45-49.
- McVey DS, Melissa K and Chengappa MM. Veterinary Microbiology, Third Edition. John Wiley & Sons, Inc. Published 2013 by John Wiley & Sons, Inc. Laboratory Diagnosis, 2013; 18.
- Miller KA, Blackall LL, Miflin JK, Templeton JM, Blackall PJ. Detection of *Helicobacter pullorum* in meat chickens in Australia. Australian veterinary journal, 2006; 84(3): 95-97.
- 40. Mohammed AA. *Effect of Escherichia coli and Citrobacter freundii on Broiler Performance and Carcass Characteristics* (Doctoral dissertation, Sudan University of Science and Technology), 2014.
- 41. MoLFD. Ministry of Livestock and Fisheries Development (1996-2007). Various Animal Production Annual Reports, 2007.
- 42. Nduthu PW. Social-economics influence on indigenous poultry production project in Kenya. A case of Machakos indigenous poultry. International Journal of Education and Research, 2015; 1(3): ISSN 2201-6333.
- 43. Nga VT, Ngoc TU, Minh LB, Ngoc VT, Pham VH, Nghia LL, Son NL, Van Pham TH, Bac ND, Tien TV, Tuan NN. Zoonotic diseases from birds to humans in Vietnam: possible diseases and their associated risk factors. European Journal of Clinical Microbiology & Infectious Diseases, 2019 Jun 1; 38: 1047-1058.
- 44. Njagi LW, Mbuthia PG, Bebora LC, Nyaga PN, Minga U, Olsen JE. Carrier status for Listeria monocytogenes and other Listeria species in free range farm and market healthy indigenous chickens and ducks. East African medical journal, 2004 Nov 17; 81(10): 529-533.
- 45. Njagi LW. Carrier status, antibiotic and disinfectant sensitivity patterns of Listeria Monocytogenes and other aerobic bacteria in scavenging chickens and ducks (Doctoral dissertation), 2003.
- 46. Osano O, Arimi SM. Retail poultry and beef as sources of Campylobacter jejuni. East African medical journal, 1999 Mar 1; 76(3): 141-144.
- 47. Permin A, Hansen JW. Epidemiology, diagnosis and control of poultry parasites. Fao, 1998.
- 48. Permin A, Pedersen G. The need for a holistic view on disease problems in free-range chickens. Network for smallholder poultry development the royal veterinary and agricultural university Frederiksberg, Denmark. 2002 Jul 1.

I

- Pohjola L. Backyard poultry flocks in Finland: an infection risk to commercial poultry or humans? Doctoral dissertation, University of Helsinki. http: //urn.fi/URN: ISBN: 978-951-51-3055-6
- Ratnam SS, March SB, Ahmed R, Bezanson GS, Kasatiya S. Characterization of *Escherichia coli* serotype O157: H7. Journal of Clinical Microbiology, 1988 Oct; 26(10): 2006-2012.
- Ravinder S, Aujla Roshan Patel. Creatine Phosphokinase. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan (Last Update: April 20, 2021). Bookshelf ID: NBK546624PMID: 3153623. 2021.
- 52. Saif YM, Barnes HJ, Glisson JR, FadlyAM M, Swayne DE. Diseases of poultry 11thed. Iowa State University Press, 2003; 56: 137-149.
- 53. Sakas PS. Understanding avian laboratory tests. Niles animal hospital and bird medical center, Milwaukee Ave. Niles. Adapted from essentials of avian medicine: a practitioner's guide. 2nd ed. by Peter S. Sakas DVM, MS. Niles, IL: AAHA Press, 2002.
- 54. Saleh I, Alwan N, Barbour E, Azhar E, Harakeh S. Listeria infections: epidemiology, pathogenesis and treatment. InListeria Infections: Epidemiology, Pathogenesis and Treatment. Nova Science Publishers, Inc, 2012; 155-166.
- 55. Sanaa M, Poutrel B, Mannered JL and Serieys. Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. Journal of Dairy Science, 1993; 76: 2891–2898.
- 56. Steubing PM. Isolation of an unknown bacterium from soil. Tested studies for laboratory teaching, 1993; 14: 81-114.
- 57. Tamiru F, Dagmawit A, Askale G, Solomon S, Morka D, Waktole T. Prevalence of ectoparasite infestation in chicken in and around Ambo Town, Ethiopia. J Vet Sci Technol, 2014 Jan 1; 5(189): 10-4172.
- Taylor MA, Coop RL, Wall RL. Veterinary parasitology. 4th ed. John Wiley & Sons; 2015 Dec 3. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews, 2015 Jul; 28(3): 603-661.
- Walker MJ, Barnett TC, McArthur JD, Cole JN, Gillen CM, Henningham A, Sriprakash KS, Sanderson-Smith ML, Nizet V. Disease manifestations and pathogenic mechanisms of group A Streptococcus. Clinical microbiology reviews, 2014 Apr; 27(2): 264-301.
- 60. Wamboi P, Waruiru RM, Mbuthia PG, Nguhiu JM, Bebora LC. Haemato-biochemical changes and prevalence of parasitic infections of indigenous chicken sold in markets of Kiambu County, Kenya. International journal of veterinary science and medicine, 2020 Jan 1; 8(1): 18-25.
- 61. Whitehead ML, Roberts V. Backyard poultry: legislation, zoonoses and disease prevention. Journal

L

of Small Animal Practice, 2014 Oct; 55(10): 487-496.

- 62. WHO. Key facts on E. coli. WHO factsheet, 2018.
- 63. Wigley P, Humphrey T and Daly J. Welfare FA, zoonotic diseases, human health and farm animal welfare. Reports on '*Salmonella* in poultry and pig production' and 'Zoonotic *Escherichia coli* in cattle production and other livestock'; '*Campylobacter* in poultry' and 'Avian Influenza' and 'Swine Influenza', 2013; 4-8.
- 64. Zander DV., Bermudez, AJ, and Mallinson, ET. Principles of disease prevention: diagnosis and control. Diseases of poultry. 10th edition, Iowa state University Press, Ames, and U S A, 1997; 37-45.
- 65. Zeryehun T, Yohannes Y. Ectoparasite infestation of free scavenging chickens reared under traditional backyard production system in Wolayita Zone, southern Ethiopia. Ethiopian Veterinary Journal, 2015 Dec 3; 19(2): 55-66.

I