

BLOOD AND GASTROINTESTINAL TRACT PROTOZOA INFECTIONS OF DOMESTICATED CHICKEN SLAUGHTERED IN LAGOS CENTRAL, SOUTHWESTERN NIGERIA.

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ABSTRACT

This study investigated the prevalence and intensity of blood and intestinal protozoa infections of domestic chickens slaughtered at the oyingbo live-bird market in Lagos State, Nigeria. Blood and intestinal samples were collected from a total of 100 birds from October through December, 2016. Thin and thick smears of blood were parasitologically screened. Mucosal scrapings and intestinal contents were examined microscopically in the laboratory for other parasitic infections using wet preparation and ziehl-neelsen acid-fast staining techniques. Parasitaemia counts were estimated. Oocysts of eimeria spp. were recorded in the various sections of the alimentary canal. Plasmodium spp. were the only haemoprotozoa encountered in the study, with a prevalence of 16%. There was no statistically significant difference in malaria infection between sexes and breeds of chickens ($p>0.05$). of the birds sampled in the study, 22 (22%) were infected with eimeria spp., while 18 (18%) had cryptosporidium spp. infection. exotic breeds had higher prevalence rate of cryptosporidium spp. infection than the local breeds ($p<0.05$). There was however no statistically significant difference in the enteric protozoa infections between sexes of the chickens ($p>0.05$). Majority of the birds (72.2%) infected with cryptosporidium spp. had unapparent infections when compared to those with severe and low-grade infections ($p<0.05$). Eimeria spp. was most prevalent in the jejunum (29.41%) and occurred least in the large intestine (8.82%). The occurrence of protozoan infections among poultry in this study suggests the need for control, so as to deliver safe and wholesome poultry products.

Keywords: Protozoa, Blood, Intestine, Prevalence, Chickens

INTRODUCTION

Parasitic protozoa infections are ubiquitous among human and animal populations, causing life-threatening diseases of public health and veterinary importance (Schmidt and Roberts, 2010; Andrews *et al.*, 2014). They are common among poultry animals and cause diseases of economic importance in the poultry industry (FAO, 2004; McDougald *et al.*, 2008; Yegani and Korver, 2008). Coccidiosis, an intestinal disease of poultry, caused by protozoa of the genus *Eimeria*, accounts for significant global economic losses estimated at over 3 billion US\$ annually (Fornace *et al.*, 2013).

The most important of these parasites belong to the phyla Apicomplexa and Sarcomastigophora exclusively, with a significant majority either parasitizing the blood or intestinal cells (McDougald *et al.*, 2008). *Plasmodium*, *Leucocytozoon* and *Haemoproteus* species are the main haemoprotozoa affecting poultry (Benedikt *et al.*, 2009). Although they are not a common cause of morbidities and mortalities, these haemosporidia have been implicated in occasional disease outbreaks and have potential to affect host fitness, feed consumption and conversion efficiencies (Momin *et al.*, 2014). Intestinal protozoa mostly reported among poultry include those of the genera *Eimeria*, *Histomonas*, *Cryptosporidium*, *Trichomonas* and *Giardia*. These parasites pose significant threats to the health, welfare and productivity of affected birds (Nagwa *et al.*, 2013; Adang *et al.*, 2014; Mohammed and Sunday, 2015).

Presently, poultry production makes significant contribution to the Gross Domestic Products of several countries, ensures food security and helps combat malnutrition (Blake and Tomley, 2014; Mohammed and Sunday, 2015). Therefore, factors limiting high productivity of poultry industries, of which parasitic diseases are most significant, must be monitored. A number of studies have reported the occurrence and prevalence of blood and intestinal protozoa among poultry in various parts of the world: Poulsen *et al.* (2000) in Ghana; Nagwa *et al.* (2013) in Egypt; Momin *et al.* (2014) in Bangladesh; including Nigeria (Luka and Ndams, 2007; Nnadi and George, 2010; Usman *et al.*, 2012; Kolade and Agbolade, 2014; Lawal *et al.*, 2016a,b). Despite this plethora of works, none have reported the situation in Lagos (Nigeria), a major financial centre in Africa.

The study therefore seeks to determine the prevalence and diversity of protozoa infections among the domestic chickens slaughtered at the Oyingbo live-bird market in Lagos State, to sensitize her poultry industry on the potential threat posed against the health, welfare and productivity of these economically important avian species.

MATERIALS AND METHODS

Study Area

This cross-sectional study was carried out in Oyingbo live-bird market situated in Ebute-Metta area of Lagos State, within Lagos Mainland Local Government Area (L.G.A.) of Lagos State, South-western, Nigeria.

Sample Collection

A total of 100 market-aged domestic chickens earmarked for slaughter at the study site were sampled from October through December, 2016. The sex (male or female) and breed (exotic or local) of each sampled bird were recorded accordingly. Blood was collected from severed veins at the neck region of the birds, into EDTA-coated bottles, at point of slaughter. The bottles were properly sealed and rolled gently to ensure complete mixture of the anticoagulant chemical, EDTA, with blood, so as to prevent clotting. The blood samples were labeled accordingly and conveyed to the laboratory for parasitological examination. Intestinal tract of selected chickens were collected immediately the birds were slaughtered and dissected, and were further divided with the aid of a scissors into five distinct segments: duodenum, jejunum, ileum, large intestine and caeca. Content and mucosal scrapings of each intestinal segment were collected into separate sample bottles, preserved with a few drops of normal saline and labeled accordingly. The samples were transported immediately for processing and microscopic examination at the Parasitology unit, Microbiology department of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

Parasitological Examination

Blood Examination

Thin and thick films of blood samples were prepared on separate clean, dry and grease-free microscopic slides, and allowed to dry completely. The thin films were fixed with absolute methanol and stained with 10% Giemsa solution for 10 minutes. The thick smears were left unfixed, but also stained in 10% Giemsa solution for 10 minutes. Excess stain was carefully washed off the slides using distilled water, and the stained films were allowed to dry. The resulting blood films were observed under the microscope at higher magnifications ($\times 40$ and $\times 100$) for the detection of haemoprotezoa (WHO, 2010).

Blood Parasitaemia Count

The 'plus system' was used to determine malaria parasite density in the thick blood films as previously described by Uneke *et al.* (2009) and WHO (2010). Parasitaemia was graded as 1-10 parasites per 100 oil-immersion thick film fields ('+'), 11-100 parasites per 100 oil-immersion thick film fields ('++'), 1-10 parasites per single oil-immersion thick film field ('+++') and >10 parasites per single oil-immersion thick film field ('++++').

Wet Preparation/Lugol's Iodine Staining

A drop of fresh intestinal samples (mucosal scrapings and luminal contents) were emulsified with few drops of physiological saline on a microscopic slide and covered with a cover slip for the detection of motile trophozoites, oocysts and/or cysts under $\times 40$ magnification of a compound microscope. This was done as described by Soulsby (1982). The number of trophozoites, oocysts and/or cysts found were identified, counted and recorded at $\times 40$ magnification. To facilitate the examination of these parasites, wet mounts of intestinal/caecal contents and scrapings were treated with a few drops of dilute Lugol's iodine solution (10.5%) (FAO, 1998).

Ziehl-Neelsen Acid-fast Staining

To demonstrate oocysts of *Cryptosporidium* species, intestinal tract samples were subjected to Ziehl-Neelsen acid-fast staining technique described by Garcia *et al.* (1983).

Counting of Oocysts

The oocyst per gram (OPG) of intestinal content/mucosal scrapping was determined by multiplying the number of oocysts in 1g of faeces by 24. Counts of oocysts less than 10 (<240 OPG), between 10 and 20 (240-480 OPG) and greater than 20 (>480 OPG) were regarded as unapparent, low-grade and severe infections respectively as described by Lawal *et al.* (2008).

Data and Statistical Analysis

Data generated were entered and managed in Microsoft Excel 2010 worksheet, and analysed using Chi-square Goodness-of-Fit Test, at 0.05 level of confidence, on SPSS 16.0 statistical software package.

RESULTS

Plasmodium spp. were the only haemoprotozoa encountered in this study, and were found to infect 16 (16.0%) of all the birds examined. No significant difference ($P > 0.05$) was observed in avian malaria infection between males (19.4%) and females (14.5%); and exotic (19.6%) and local (12.2%) breeds (Table 1).

Of the 100 chickens sampled in this study, 22 (22.0%) and 18 (18%) were positive for *Eimeria* and *Cryptosporidium* species respectively. The prevalence of these enteric protozoa, based on the sex and breed of sampled birds is presented in Table 1. There was no significant difference ($P > 0.05$) in the prevalence of *Eimeria*

spp. infections between sexes and breeds of chickens. *Cryptosporidium spp.* infection was however significantly higher ($P < 0.05$) among exotic (25.5%) than local (10.2%) species, although statistically similar ($P > 0.05$) when prevalence was compared between sexes of the birds.

Table 2 shows the loads of *Plasmodium spp.* infection among the avian malaria positive chickens. 1-10 parasites per 100 thick film fields were recorded among majority of the infected birds (75.0%). 1-10 ('+') and 11-100 ('++') malaria parasite counts per 100 thick film fields were recorded mostly among hens than in cocks and in exotic breeds than among the local species.

Table (1): Prevalence of blood and gastro-intestinal protozoa infections by breed and sex of chicken slaughtered at Oyingbo market

Criteria	Total No. examined	No. infected (%)		
		<i>Plasmodium spp.</i>	<i>Eimeria spp.</i>	<i>Cryptosporidium spp.</i>
Breed				
Exotic	51	10 (19.6)	11 (21.6)	13 (25.5)*
Local	49	6 (12.2)	11 (22.4)	5 (10.2)
Total	100	16 (16.0)	22 (22.0)	18 (18.0)
Sex				
Male	31	6 (19.4)	6 (19.4)	3 (9.7)
Female	69	10 (14.5)	16 (23.2)	15 (21.7)
Total	100	16 (16.0)	22 (22.0)	18 (18.0)

* represents significant difference at $P < 0.05$

None of the chickens infected with *Plasmodium spp.* in this study however had 1-10 or greater than 10 parasites per single thick film fields.

Of the birds infected with *Eimeria spp.*, 14 (63.6%) had severe infections, while 7 (31.8%) and 1 (4.5%) were diagnosed with unapparent and low-grade infections respectively.

Table (2): Intensity of *Plasmodium spp.* infections among slaughtered chicken in Oyingbo market

Intensity grade	Sex [n(%)]			Breed [n(%)]		
	Male	Female	Total	Exotic	Local	Total
‘+’	5 (41.7)	7 (58.3)	12 (100.0)	7 (58.3)	5 (41.7)	12 (100.0)
‘++’	1 (25.0)	3 (75.0)	4 (100.0)	3 (75.0)	1 (25.0)	4 (100.0)
‘+++’	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
‘++++’	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Where ‘+’ = 1-10 parasites per 100 thick film fields, ‘++’ = 11-100 parasites per 100 thick film fields, ‘+++’ = 1-10 parasites per single thick film field and ‘++++’ = >10 parasites per single thick film field.

Higher proportions of the severely and unapparently infected birds were females when compared with the males. There was no difference in these infection grades between the chicken breeds. Unapparent infections (72.7%) were most common among chickens infected with *Cryptosporidium spp.*, while 4 (22.2%) and 1 (5.6%) of these birds had low-grade and severe infections respectively. Female (76.9%) and exotic (61.5%) chickens had higher proportion of unapparent *Cryptosporidium spp.* infections when compared with their respective counterparts (Table 3).

Table (3): Intensity of gastro-intestinal protozoa infections according to breed and sex of the chicken slaughtered at Oyingbo market

Criteria	<i>Eimeria spp.</i> [n(%)]			<i>Cryptosporidium spp.</i> [n(%)]		
	Unapparent	Low-grade	Severe	Unapparent	Low-grade	Severe
Sex						
Male	1 (14.3)	1 (100.0)	4 (28.6)	3 (23.1)	0 (0.0)	0 (0.0)
Female	6 (85.7)	0 (0.0)	10 (71.4)	10 (76.9)	4 (100.0)	1 (100.0)
Total	7 (100.0)	1 (100.0)	14 (100.0)	13 (100.0)	4 (100.0)	1 (100.0)
Breed						
Exotic	4 (57.1)	0 (0.0)	7 (50.0)	8 (61.5)	4 (100.0)	1 (100.0)
Local	3 (42.8)	1 (100.0)	7 (50.0)	5 (38.5)	0 (0.0)	0 (0.0)
Total	7 (100.0)	1 (100.0)	14 (100.0)	13 (100.0)	4 (100.0)	1 (100.0)

Figure 1 illustrates the occurrence of *Eimeria spp.* across the intestinal segments of the infected birds. Oocysts were recovered mostly from the jejunum of chickens (29.4%), followed by the ileum (26.5%), duodenum (20.6%), caeca (14.7%), and least in the large intestine (8.8%). There was no statistically significant difference in the occurrence of the coccidian across these segments ($P>0.05$).

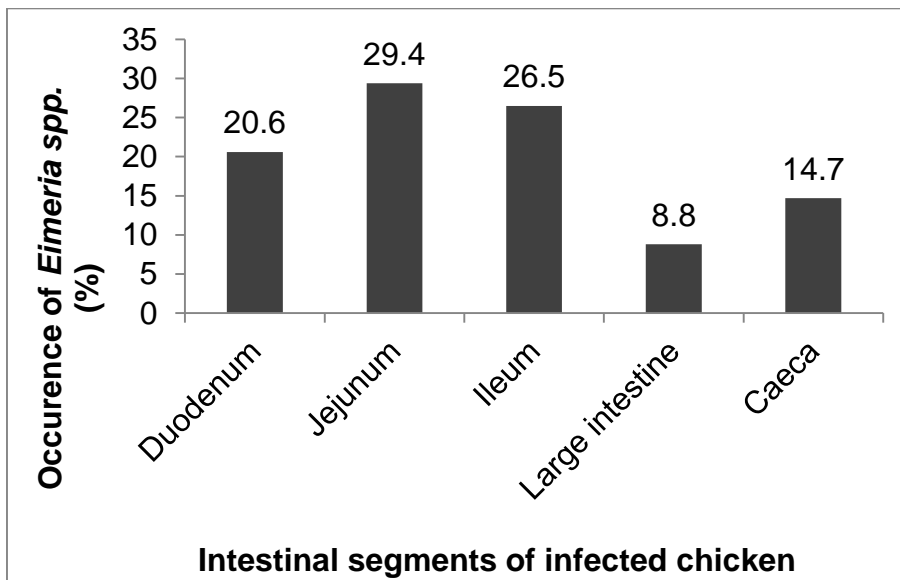


Figure (1): Distribution of *Eimeria spp.* infection across the gastro-intestinal tract of the chickens slaughtered at Oyingbo market

DISCUSSION

Parasitic diseases are one of the major factors limiting poultry production; they are responsible for significant economic losses recorded by poultry sectors worldwide (Dube *et al.*, 2010). The most important of these diseases are caused by protozoans, especially those which parasitize the blood and intestinal tract of poultry (McDougald, 1998; Momin *et al.*, 2014).

Plasmodium spp. were identified as the only haemoprotozoa infecting poultry sampled in this study, occurring at a prevalence rate of 16.8%. Considerable similar prevalence rates were reported by Usman *et al.* (2012) (12%) and Lawal *et al.* (2016a) (8%) in Sokoto and Bornu states (in Nigeria) respectively. The low infection rate however encountered in this study, in addition to the absence of other blood protozoan parasites which had been previously reported among chickens in Bornu and Imo states in Nigeria by Lawal *et al.* (2016a) and Opara *et al.* (2014) respectively, might be due to the fact that the present investigation was carried out in the dry season, a period characterized by low abundance of the suitable insect vectors responsible for the transmission of these parasites. Furthermore, this study supports claim of some researchers that infection with avian malaria parasites may not be associated with the gender or breed of chicken (Sabuni *et al.*, 2011; Opara *et al.*, 2014 and Lawal *et al.*, 2016a).

Significant majority of the chickens infected with *Plasmodium spp.* in this study had low parasitaemia. The implication of this finding, in relation to pathology, health and productivity of the birds cannot be explained because of paucity of information on the pathogenicity and virulence of the malaria parasite species infecting poultry. This therefore necessitates the need to carry out more studies that provide adequate information on the biopathological characteristics of the various species of *Plasmodium* found infecting poultry, and on the severity of the diseases they cause, in relation to infection intensity. Also, low malaria intensity diagnosed for most of the infected birds could be related to their immune status.

Eimeria and *Cryptosporidium* species were the only protozoa found parasitizing the gut of the birds in this survey. This finding supports the claim that these coccidia are the most common intestinal parasites of poultry (McDougald, 1998). Nagwa *et al.* (2013) also identified these parasites as the only protozoa inhabiting the intestines of various poultry species sampled in Egypt.

The prevalence rate of *Eimeria spp.* infection (22%) recorded in this study was lower than those determined in other surveys conducted during the rainy season in

Kaduna (33.3%) and Abuja (34.5%) states by Jatau *et al.* (2012) and Jegede *et al.* (2015) respectively. This observed difference is expected because dry seasons, the period in which this current study was carried out, are characterized by relatively low humidity which has been identified to be disadvantageous to *Eimeria* oocyst sporulation, therefore hindering transmission and survival of the protozoa among poultry flocks.

Majority of the exotic breeds of chicken, when compared with their local counterparts, were infected with *Cryptosporidium spp.* Such finding was reported by Lawal *et al.* (2016b) for *Eimeria spp.* infections, and was attributed to certain breed factors which might be present in these chicken breeds. There was however no influence of sex of the sampled birds on the frequency of occurrence of both enteric protozoa encountered in this study. This finding is similar to that reported by Jegede *et al.* (2015) for chickens reared within a guinea savannah zone in Abuja, Nigeria.

The dosage or amount of infective oocysts a susceptible bird ingests at a time is one of the significant factors considered in the diagnoses of the severity of coccidiosis (McDougald *et al.*, 2008). In this present study, a significantly high percentage of the infected chickens were severely infected with *Eimeria spp.* This is a strong indication of potential losses among these poultry species in the study area, as death normally follows in most chickens that are severely infected (Dickinson, 1942). This finding was however not in consonance with findings of another study carried out in Zaria, Nigeria where unapparent *Eimeria spp.* infection was high and most prevalent among chickens (Jatau *et al.*, 2012). This discrepancy can be attributed to the levels of infective oocysts contamination in the environment where these studies were conducted.

Unapparent infections of *Cryptosporidium spp.* were significantly higher among the chickens when compared to other intensity grades. This finding might be of economic significance to the poultry industry, as sub-clinical coccidiosis, caused by unapparent infections of *Eimeria* is often manifested by poor performance, impaired feed conversion, poor flock uniformity and poor growth of poultry (Mohammed and Sunday, 2015).

CONCLUSION

This study revealed *Plasmodium spp.* as the only protozoa parasitizing the blood of chicken in the study area, and reported the occurrence of intestinal infections caused by protozoa belonging to the genera *Eimeria* and *Cryptosporidium* exclusively. The relatively low prevalence rates of these parasitic infections is

attributed to the period (dry season) within which this current survey was conducted. Furthermore, high percentages of severe and unapparent intestinal infections of *Eimeria spp.* and *Cryptosporidium spp.* respectively were diagnosed among majority of the birds infected with these parasites. Such finding might be of economic significance to the poultry industry, as severe and sub-clinical intestinal infections are known to negatively affect bird welfare, health and productivity. Poultry farmers and traders are therefore encouraged to adopt standard biosecurity plans and adhere to good poultry management practices necessary for the control of these infections among poultry, so as to avert losses due to these parasites in the local poultry industry.

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