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Prevalence of Avian Haemosporidian Parasites in Village Chickens (*Gallus gallus domesticus*) from Kwami, Gombe State, Nigeria

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ABSTRACT

This study was carried out to determine the prevalence of avian haemosporidian parasites in village chickens in Kwami, Gombe State, Nigeria. Blood samples were collected from 346 apparently healthy village chickens in Kwami Local Government Area of Gombe State, and a total of 45 farmers were interviewed using structured questionnaires. Microscopy revealed an overall prevalence of 20.8% (72/346) for haemosporidian parasites comprising of *Plasmodium* spp. with 41 (11.8%; 95% CI = 8.9 – 15.7) and *Haemoproteus* spp. 23 (6.6%; 95% CI = 4.5 – 9.8) as single infection and mixed infection of *Plasmodium* + *Haemoproteus* spp. having 8 (2.3%; 95% CI = 1.2 – 4.5). Prevalence of avian haemosporidian parasites was significantly higher in male (13.9%) compared to hens (6.9%), as well as in adults (14.5%) compared to growers (6.4%). The prevalence was also found to be higher in the rainy season (15.6%) compared to the dry (5.2%) seasons of the study period. Questionnaire survey on attitude and practice of the village chicken farmers in the study area has shown that chickens are raised under the extensive management system and are reared with other village poultry species on free range. Inadequate biosecurity, poor management and husbandry systems constituted the risk factors associated with haemoparasite infections in this study.

Keywords: Avian Malaria; Gombe State; Haemosporidian parasites, Microscopy, Scavenging Chickens

INTRODUCTION

Large population of rural and semi- rural areas of the world rear poultry, which plays a significant economic role in providing high animal protein (meat and eggs) for food security, alleviates poverty by providing work opportunities and income (Otte, 2006). Small scale poultry production system is mostly characterized by holding small number of birds that spend more time scavenging around the surrounding and not receiving any special veterinary attention (Takele and Oli, 2011). They are also not provided with modern housing facilities for roosting at night and are permitted to breed naturally.

Diseases are considered as a major challenge to successful village chicken production system in developing countries (Hamer *et al*., 2013; Sehgal, 2015). Most reported investigations of infectious poultry diseases in developing countries in Africa centered on viral, bacterial, protozoan diseases, while others focused on ectoparasites and gastrointestinal parasites (Letebrhan *et al*., 2015; Weyuma *et al*., 2015). Infections of haemoparasites and their consequences in chickens had received little attention. Village chickens are typically reared under the extensive management systems in most parts of Nigeria (Opara *et al*., 2014). They have access to the environment where they scavenge for food even on unhygienic garbage dumps and near unhygienic pool of water (Chepkemoi *et al*., 2017). When compared to chickens reared in screened pens or under intensive management systems, this makes them more susceptible to bites from a variety of haemophagus arthropod vectors that may harbour blood parasites (Sehgal *et al*., 2011; Letebrhan *et al*., 2015; Malatji *et al*., 2016). Although previous studies have identified blood parasites primarily in wild and other birds in tropical areas worldwide, recent studies have found emerging haemosporidian parasite infections in poultry species including chickens (Abdul Momin *et al*., 2014; Tostes *et al*., 2015; Opara *et al*., 2016). Plasmodium, Leucocytozoon, Haemoproteus, Microfilaria, Aegyptinella, Fallisia and Trypanosoma species are genera of haemoparasites recorded in chickens around the world (Valkiûnas, 2005; Braga *et al*., 2011; Dimitrov *et al*., 2014; Gimba *et al*., 2014). Clinical disease in infected birds has been reported to be associated with fever, depression, anorexia, body weight loss, dyspnea, hepatomegaly, splenomegaly and ocular haemorrhage (Dunn *et al*., 2011;

Knowles *et al*., 2011). Severe haemosporidian parasite infections in domestic and wild birds can lead to death, involving various symptoms such as anaemia, thrombocytopenia and inflammation (Valkiûnas *et al*., 2005; Naqvi *et al*., 2017). Infections of chickens with avian haemosporidian parasites have been reported in a few investigations from Nigeria (Lawal *et al*., 2016; Opara *et al*., 2016; Hassan *et al*., 2018; Ogbaje *et al*., 2019). The current level of research and knowledge regarding avian haemosporidian parasites in Nigeria is low; hence increased efforts are required to understand the region's diversity, prevalence and distribution of haemosporidian parasites in the country (Ogbaje *et al*., 2019). In Kwami Local Government Area of Gombe State, there is availability of dense vegetation, which could provide suitable ecosystem for the breeding of diverse arthropods capable of spreading haemosporidian parasites to chickens. Hence, a study of the prevalence of haemosporidian parasite infections in village chickens is required to serve as an input in planning strategies for effective control measures in the study area.

MATERIALS AND METHODS

Study area

This study was carried out in Kwami Local Government Area of Gombe State, Nigeria. It has an area of 1,787 km2 (690 sq mi) and a population of 195,298 at the 2006 census. The mean temperature varies from 30 – 320C, the rainfall cycle is unimodal between 700 – 1250 mm and is characterized by distinct dry seasons (October-May) and rainy seasons (June-September).

Study Design

A cross-sectional study design using convenient sampling techniques was used to sample three (3) out of the total nine available districts, viz: Doho, Kwami and Mallam Sidi, these three districts were selected based on accessibility and willingness of respondents to participate in the study. The study was conducted within a period of 10 months between November, 2016 and September, 2017.

Study Population

This study sampled a total of 346 chickens (*Gallus gallus domesticus*) of both sexes (cocks and hens). Chickens sampled were classified as growers (3–4 months) and adults (over 5 months) according to Addass *et al*. (2012) age descriptions for chickens. Chickens are mainly village chickens within the study area and were sampled from households that raise different poultry species. Blood samples were collected from apparently healthy live chickens after receiving consent from their owners.

Blood Sample Collection

Using sterile 5ml syringes and 23 gauge needles, about 2 – 3ml of blood samples were aseptically collected from each sampled chicken via the wing vein (venipuncture). Blood samples were immediately dispensed into sample bottles containing EDTA as anticoagulant. The collected blood samples were stored at 40C and transported to the Department of Veterinary Parasitology and Entomology Research Laboratory, University of Maiduguri, Nigeria for parasitological procedures. The period of blood samples collection was considered as rainy and dry seasons.

Blood Smear Preparation

Thin blood and buffy coat smears were made on a clean dry slide according to standard protocol as described by Mello *et al*. (2014), smears were allowed to air dry for a few minutes, then fixed in absolute methanol, and then allowed to air dry again, before properly labelling each slide.

Microscopic Detection of Haemosporidian Parasites

The slides were stained with Giemsa stain (pH 7.2), rinsed with distilled water, and allowed to air dry according to the standard procedures described by Thrall (2004) and Ribeiro *et al*. (2005). Stained blood smears were later viewed under a light Olympus® (Japan) microscope first at low magnification (40x), and then at high magnification (100x) oil immersion objective for the presence of intracellular blood parasites. The haemosporidian schizonts, gametocytes, and trophozoites were examined and identified based on morphology as previously described by Valkiûnas (2005). Photomicrographs were taken using a digital camera 20.1 MP (Sony, Tokyo, Japan DSC-W800/B 20 Megapixel) and the printed copies were compared to standard plates (Taylor *et al*., 2007).

Questionnaire Survey

To gather some information regarding associated risk factors, a structured questionnaire was administered to interview chicken farmers within the study area to assess some predisposing factors to haemosporidian parasites infection among scavenging village chicken flocks. The criteria considered in the questionnaire interview included the chickens feeding patterns, type of husbandry and management system.

Data Analysis

Data analysis was performed using GraphPad Prism software (GraphPad Inc., San Diego, CA). Prevalence rates were calculated as percentages of proportion, Chi-squared test was used to compare categorical variables (age, sex and season). Analysis for the odds ratios was estimated using the GraphPad Prism software, calculation of the lower and upper limits of the 95% confidence interval (CI) for a proportion was done according to the methods described by Newcombe (1998). Differences were considered significant for p-values equal to or less than 0.05.

RESULTS

Table 1 summarizes the results of the prevalence of avian haemosporidian parasites infections in village chickens from Kwami, Gombe State, Nigeria. Out of the total of 346 chickens sampled and examined, haemosporidian parasites were found in 72 chickens with an overall infection rate of 20.8% (95% CI = 16.9% - 25.4%). The prevalence rate in Mallam Sidi (16.4%; 95% CI = 10.6% – 24.4%) was found to be higher followed by Kwami (25.2%; 95% CI = 18.7% – 33.7%) and Doho (20.5%; 95% CI = 14.2% – 28.7%) districts of the study area. However, chi-squared test showed no significant difference in the infection rate based on district ($\chi 2 = 1.786$; df = 2; p = 0.4093).

The types of haemosporidian parasites in village chickens from Kwami, Gombe State, Nigeria is summarized in Table 2. The prevalence of *Plasmodium spp*. infection (11.8%; 95% CI = 8.9% – 15.7%) is higher compared to *Haemoproteus spp*. infection (6.6%; 95% CI = 4.5% – 9.8%). Thus, *Plasmodium spp*. and *Haemoproteus spp*. mixed infections (2.3%; 95% CI = 1.2% – 4.5%) were found to be the least prevalent in this study period (Figures 1 and 2).

Table 3 summarizes the results of some risk factors associated with haemosporidian parasites infection among village chickens in Kwami, Gombe State, Nigeria. The prevalence of haemosporidian parasite infections in males (13.9%; 95% CI = 10.6% – 17.9%) was found to be higher than in females (6.9%; 95% CI = 4.7% – 10.1%) chickens, and the association between haemosporidian parasites prevalence and age of chickens was found to be statistically significant ($p = 0.0040$; $\chi2 = 8.300$; Odd ratio = 0.4384). However, the prevalence of haemosporidian parasites was also found to be higher in adults (14.1%; 95% CI = 11.1% – 18.6%) compared to grower chickens (6.4%; 95% CI = 4.2% – 9.4%) and the association between the prevalence of haemosporidian parasites infections and age group of chickens was also found to be statistically significant ($p< 0.0017$; $\chi2 = 9.848$; Odd ratio = 0.4031). The prevalence of haemosporidian parasites in the rainy period (15.6%; 95% CI = 12.2% – 19.8%) was found to be higher compared to the dry period (5.2%; 95% CI = 3.3% – 8.1%) of the sampling period; and the association between the prevalence of haemosporidian parasites infections and sampling period was also found to be statistically significant ($p< 0.0001$; $\chi2 = 21.485$; Odd ratio = 0.2559).

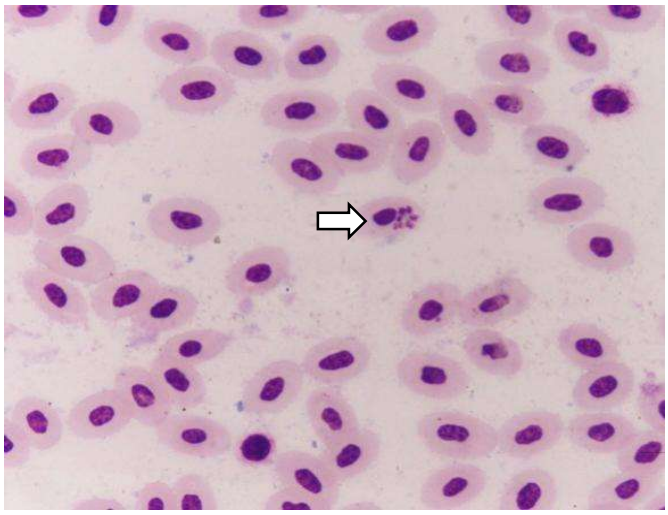

Figure 1: Chicken blood smear showing RBC infected with *Plasmodium* species. Pigmented gametocytes are present within the cytoplasm of mature erythrocytes (arrow)

The result of poultry farmers' responses on the chickens' husbandry and management systems in Kwami, Gombe State Nigeria is shown in Table 4. Out of 45 respondents, 45 (100.0%) poultry farmers revealed that their chickens were exclusively reared under the extensive management system, while none 0 (0.0%) of the poultry farmers in the study area rears chickens under the intensive management system. Out of 45 respondents, 33 (73.3%) poultry farmers revealed that their chickens usually scavenge for feeds on refuse dumps, near stagnant pools of water and other unhygienic places while 32 (26.7%) revealed that their chickens are usually restricted from scavenging on refuse dumps and near stagnant pools of water. There was a significant difference ($p< 0.05$) between poultry farmers whose chickens scavenge on refuse dumps and near stagnant pools of water and those that do not. Considering poultry farmers' responses on rearing of chickens with other domestic birds in the study area, out of 45 respondents, 30 (66.7%) respondents revealed that they rear their chickens with other domestic birds such as ducks, turkeys, guinea fowls and pigeons, while 15 (33.3%) respondents revealed that they do not rear their chickens with other domestic birds. There was a significant difference ($p< 0.05$) between proportion of respondents that rear chickens with other domestic birds and those that do not.

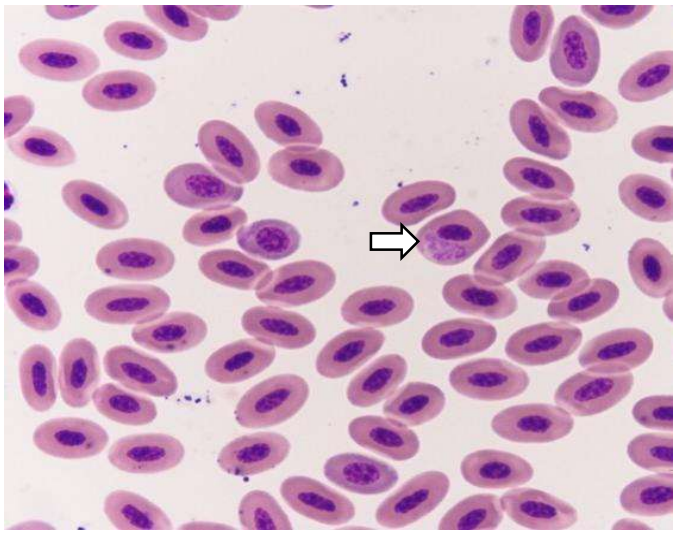

Figure 2: Chicken blood smear showing RBC infected with *Haemoproteus species*. Pigmented gametocytes curving around the nucleus of a mature erythrocyte (arrow)

DISCUSSION

The overall prevalence of haemosporidian parasites in village chickens from the present study was found to be 20.8% (95% CI = 16.9 – 25.4). This finding is lower than 46.7% and 37.7% reported from Imo and Nassarawa by States, Nigeria by Opara *et al.* (2016) and Hassan *et al.* (2018) respectively. However, this result is higher than 12.0%and 23.2% reported in Sokoto and Benue States in Nigeria by Usmana *et al*. (2012) and Ogbaje *et al*. (2019) respectively. The finding of the present study is also lower than 79.2 % reported from Kenya (Sabuni *et al*., 2011), 79.1 % in Malawi (Lutz *et al*., 2015) and 43.4 % in Ethiopia (Etisa *et al.*, 2017). Variations in the reported prevalence rates might be attributed to differences in sample sizes, season of sample collection and abundance of appropriate arthropod vectors.

The prevalence of haemosporidian parasites in chickens was found to be 25.2% in Mallam Sidi, followed by Kwami (20.5%) and Doho (16.4%). However, chi-squared test showed no significant difference in the infection rate based on district ($\chi^2 = 1.786$; df = 2; p = 0.4093).

Table 1: Prevalence of Haemosporidians Parasites in Village Chickens from Kwami, Gombe State, Nigeria

Study Areas	No. of Chickens Examined	No. of Chickens Infected	Prevalence (%)	95% CI LL – UL
Doho	110	18	16.4	10.6 – 24.4
Mallam Sidi	119	30	25.2	18.7 – 33.7
Kwami	117	24	20.5	14.2 – 28.7
Overall	346	72	20.8	16.9 – 25.4

Key: LL= Lower limit; UL= Upper limit; CI= Confidence Interval

Table 2: Type of Haemosporidian Parasites in Village Chickens in Kwami, Gombe State, Nigeria

Type of Infection	Haemosporidians Encountered	No. of chickens infected N = 346	Prevalence (%)	95% CI LL – UL
Single	*Plasmodium* spp.	41	11.8	8.9 – 15.7
	Haemoproteus spp.	23	6.6	4.5 – 9.8
Mixed	*Plasmodium* + *Haemoproteus* spp.	8	2.3	1.2 – 4.5
Overall		72	20.8	16.9 – 25.4

Key: LL= Lower limit; UL= Upper limit; CI= Confidence Interval

Table 3: Risk factors associated with Haemosporidian Parasites infections in Village Chickens in Kwami, Gombe State, Nigeria

Risk factors	No. of chickens examined	No. of chickens infected (%)	Prevalence (%) 95% CI (LL – UL)	*p*-value	χ^2	Odd Ratio
Sex						
Male	176	48 (27.3)	13.9[a] (10.6 – 17.9)	0.0040	8.300	0.4384
Female	170	24 (14.1)	6.9[b] (4.7 – 10.1)			
Age (months)						
Adults (> 5)	181	50 (27.6)	14.5[a] (11.1 – 18.6)	0.0017	9.848	0.4031
Grower (3 – 4)	165	22 (13.3)	6.4[b] (4.2 – 9.4)			
Period						
Rainy	173	54 (31.2)	15.6[a] (12.2 – 19.8)	< 0.0001	21.485	0.2559
Dry	173	18 (10.4)	5.2[b] (3.3 – 8.1)			

NB: Values with different superscripts [a,b] are significantly ($p< 0.05$) different
Key: LL= Lower limit; UL= Upper limit; CI= Confidence Interval; χ^2 = Chi-square

This finding indicates abundance of vectors capable of transmitting haemosporidian parasites to village chickens in this study area and might be connected to the fact that Mallam Sidi is the swampiest among the study areas with abundance of thick vegetation which may provide suitable ecosystem for the breeding of different arthropods especially the mosquitoes. The availability of suitable vectors coupled with the scavenging nature of village chickens, may increase their predisposition to haemosporidian parasites infections. This finding is consistent with the findings of Kar *et al*. (2014), who found that marshy residents have a role in providing favorable conditions for breeding arthropods capable of spreading haemosporidian infections and other arthropod-borne diseases to susceptible hosts.

Table 4: Village Chickens Husbandry and Management System in Kwami, Gombe State, Nigeria

Variables	Response	Study Locations (N (%))			Total Number of Respondents N = 45
		Mallam Sidi n = 15	Doho n = 15	Kwami n = 15	
Village chicken Husbandry system	Extensive	15 (100)	15 (100)	15 (100)	45 (100)
	Intensive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rear Village chickens with other poultry species	Yes	11 (73.3)	9 (60.0)	13 (86.7)	33 (73.3)
	No	4 (26.7)	6 (40.0)	2 (13.3)	12 (26.7)
Village chickens scavenge on refuse dumps	Yes	10 (66.7)	8 (53.3)	12 (80.0)	30 (66.7)
	No	5 (33.3)	7 (46.7)	3 (20.0)	15 (33.3)

Key: N = Total number of respondents; n = number of respondents in each study location

The two genera of haemosporidian identified among chickens in the present study are *Plasmodium* and *Haemoproteus* species. These two haemosporidian parasites have been reported by Gimba *et al*. (2014) in Selangor, Malaysia, Nourani *et al*. (2018) from the East of Iran and Nakayima *et al*. (2019) in Northwestern Uganda suggesting that these species of haemosporidian parasites are the most prevalent in village chickens and are worldwide in distribution.

Plasmodium species (11.8 %) was the most prevalent compared to *Haemoproteus* species (6.6%) in the present study which supports the findings of Etisa *et al.* (2017) and Nakayima *et al*. (2019). However, Opara *et al*. (2016) and Hassan *et al*. (2018) revealed *Haemoproteus* species as the most prevalent haemosporidian parasites infections among chickens in a similar study. The probable reasons for these discrepancies could be the difference in the ecologic and climatic factors, abundance of vectors and different chickens' management systems. A mixed *Plasmodium* and *Haemoproteus* species infection were also found in some infected chickens at a prevalence rate of 2.3%. This finding buttresses the finding of Naqvi *et al*. (2017) who also reported mixed *Plasmodium* and *Haemoproteus* species infection in scavenging chickens. However, the finding of the present study is lower than 47.4% reported by Hasson (2015), but higher than 0.5% reported by Nath and Bhuiyan (2017). The difference in the reported prevalence rates of mixed *Plasmodium* and *Haemoproteus* species infections in chickens may partly be attributed to variation in geographic distribution of arthropod vectors.

In the present study, a higher prevalence of haemosporidian parasites infections was observed in males (13.9%) than in females (6.9%) chickens, and the difference was statistically significant ($p< 0.05$). This finding might be attributed to availability of larger comb and wattle in male chickens that can provide abundant blood sucking site for arthropod vectors. This is consistent with the findings of Opara *et al*. (2016), Etisa *et al*. (2017), Hassan *et al*. (2018) and Ogbaje *et al*. (2019) who have also reported higher prevalence of haemosporidian parasites infections in cocks compared to hens of village chickens. However, Hasson (2015) and Naqvi *et al*. (2017) reported higher prevalence of haemosporidian parasites infection in female than in male chickens, which is contrary to the results of the present study.

The higher prevalence of haemosporidian parasites infections in adult (14.5%) compared to young (6.4%) chickens agrees with the findings of Abdul Momin *et al*. (2014), Nafyad *et al*. (2015) and Etisa *et al*. (2017), but disagrees with the findings of Sabuni *et al*. (2011) and Naqvi *et al.* (2017) who reported higher prevalence in young than in adult chickens. According to Yeshitila *et al*. (2011) higher prevalence of haemosporidian parasites infections in adult compared to young chickens could be associated with the longer periods of exposure to arthropod vectors. Adult chickens also have more prominent and well-developed combs and wattles that provide abundant sites for blood sucking arthropod vectors (Abdul Momin *et al*., 2014).

The present study reported higher prevalence of haemosporidian parasites in the rainy (15.6%) than in the dry (5.2%) season of the period of sample collection, and the difference was also statistically significant ($p< 0.05$). This could be associated to the rainy season being the most suitable season which provides optimum temperature and humidity for the proliferation of most arthropod vectors, such as mosquitoes. This finding of the present study is consistent with Igbokwe *et al*. (2008) who also reported higher prevalence of haemosporidian parasites during the rainy season, but contrasted with Nath and Bhuiyan (2017) who reported higher prevalence of haemosporidian parasites in dry season.

The result of the present study found that poultry farmers in the study area raise their village chickens under the extensive management system. In the semi-intensive or intensive management systems, chickens might be provided with some forms of special attention with regards to the provision of feed supplements, vaccination and other preventive measures, which are lacking in the extensive management system. Chickens raised under the extensive management

system are allowed to scavenge searching for food. This practice could predispose chickens to several disease agents and vectors, which concurs with Wang *et al*. (2013) who reported that free range birds have higher probability of coming in contact with various infections.

The result of this study also found that large population of poultry farmers in the present study area practice mixed rearing of village chickens with other domestic birds such as turkeys, guinea fowls and pigeons. It was observed that these poultry species fed and roost in the same place with the village chicken. The result supports Sai'du *et al*. (2004) who also reported that it is common to find a combination of different poultry species being kept in the same compound in Nigeria. According to Malann *et al*. (2016) and Kebede *et al*. (2017), mixed poultry farming may partly serve as a source of cross infection amongst the poultry species and could maintain cycles of infections indefinitely including parasitic diseases.

The result of the present study revealed that chickens in the study area during the course of free roaming usually scavenge for feeds on refuse dumps, near stagnant pools of water and other unhygienic places. This positively correlated with the occurrence of microscopically detected haemosporidian parasites in village chickens in the present study area. The refuse dumps and stagnant pools of water are no doubt the usual site for breeding of many species of arthropods which are capable of transmitting various species of parasites to chickens. The affected birds during scavenging may accidentally get bitten by haemosporidian parasites infected vectors and thereby gets infected. Proximity to water bodies has been known to be a major predictor for avian haemosporidian parasite infections prevalence in birds in most countries of the world (Mendenhall *et al*., 2013; Krama *et al*., 2015).

Conclusion

In conclusion, this present study revealed that haemosporidian parasites of chickens including *Plasmodium* and *Haemoproteus* species, are prevalent (20.8%; 95% CI = 16.9 – 25.4) in Kwami, Gombe State, Nigeria. The prevalence of haemosporidian parasites were significantly higher in male, adult chickens, and higher in the rainy season of the study period. *Plasmodium* spp. which has been reported worldwide as the cause of avian malaria in birds was found to be the most prevalent haemosporidian parasite in chickens in the study area. Questionnaire survey revealed inadequate biosecurity, poor husbandry and management practices in rearing chickens in study area.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors Contribution

UII, AAB and HIM designed and supervised the work. JRL carried out the study, collected and analyzed data, and prepared the draft manuscript. All authors have read and approved the final manuscript.

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