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Research Article

Current Status of Haemosporidian Parasites Infection in Scavenging Chickens (*Gallus gallus domesticus*) and its Awareness Amongst Village Poultry Farmers in Dukku, Gombe State, Nigeria

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Abstract

The aim of this study is to detect and determine the current status of avian haemosporidian parasites infecting scavenging chickens in Dukku, Gombe State, Nigeria. To achieve this objective, blood samples were obtained from 370 scavenging chickens from households within the study areas, thin blood film and buffy coat smears were examined using microscopy for avian haemosporidian parasites, and identification of parasites was based on their morphology. The present study showed an overall prevalence rate of 21.9%. *Plasmodium* was detected in 59 while *Haemoproteus* in 15 chickens in a single infection with prevalence rates of 15.9% and 4.1% respectively, mixed *Plasmodium* and *Haemoproteus* was found in 7 (1.9%) infected chickens. The prevalence of avian haemosporidian parasites was higher in chickens sample from Gombe Abbah (9.2%) district compared to chickens from Dukku (7.6%) and Hashidu (5.1%) districts. The prevalence of avian haemosporidian parasites was significantly higher (*P*-value: 0.0061; $\chi^2 = 7.525$; OR= 2.100) in males (14.3%) compared to females (7.6%) chickens; significantly higher (*P*-value: < 0.0001; $\chi^2 = 17.784$; OR = 2.291) in adults (16.5%) compared with grower (5.4%) chickens; and significantly higher (*P*-value: < 0.0001; $\chi^2 = 46.090$; OR = 7.690) in the rainy (18.4%) compared to dry season (3.5%) during the study period. The present study showed that village chickens farmers in the study area are unaware of avian haemosporidian parasites infection amongst their chickens' flocks, thus it is recommended that awareness campaign to educate the farmers on how to improve management practices is necessary so as to prevent widespread transmission of the parasites to commercial exotic poultry farms.

Keywords: *Avian Haemosporidian Parasites; Scavenging Chickens; Plasmodium; Haemoproteus*

Introduction

The village poultry production system is an integral part of many rural livelihoods in most underdeveloped and developing countries around the world, particularly in countries with high levels of poverty and hunger threatening their populations [1,2]. The world economy

has recently been affected by the global pandemic of the highly contagious corona virus (COVID-19) [3]. Village poultry is primarily grown for the production of eggs and meat, providing high-quality animal protein to save people from hunger that may result from the strategies of the total lockdown government policy imposed on people as one of the measures to curtail the spread of the corona virus in the world. Meanwhile, investment in the village poultry business may also create employment opportunities for young people and women as a source of income in the form of small cash from the sale of eggs and live birds [4-7]. The scavenging chickens could also be considered useful as biological pest control and used as sacrifices during both religious and traditional festivals [8]. Among the village poultry species, the chickens popularly known as the village chickens, local chickens or rural chickens predominate in numbers and popularity in the rural poultry sector [9,10]. The production system of village chickens in African countries faces several challenges, including infectious disease threats, which are considered to be the most important limiting factor for the productivity of village chickens in Africa, including Nigeria [11,12]. Scavenging chickens have been documented to be susceptible to infectious parasitic diseases and also serve as host for most parasites, including helminthes, ectoparasites, protozoan and haemoparasites [13-17]. They may play a significant role in the transmission and maintenance of these diseases among avian population [18].

Haemosporidian genera such as *Plasmodium*, *Haemoproteus* and *Leucocytozoon* species has been reported with variable prevalence amongst scavenging chickens in most part of the world including Nigeria [19,20]. Other genera of haemoparasites such as *Trypanosoma, Anasplasma; Babesia, Atoxoplasma*, *Hepatozoon* and *Microfilariae* have also been reported in birds which were found sharing close proximities with the scavenging chickens [21]. Haemoparasites that causes avian malaria may appear in apparently healthy bird with mild or no apparent clinical manifestation [22]. However, infection may lead to immune-suppression which may cause other negative health impacts on infected birds [23,24]. Variable prevalence of haemoparasites which cause avian malaria infection in scavenging chickens has been correlated with the availability and abundance of suitable arthropod vectors and other evidence associated with influx of migratory wild birds [25,26]. However, previous studies have shown that the extensive management system employed in rearing village chickens is one of the most important predisposing factors of avian malaria amongst scavenging chickens in developing countries including Nigeria [27].

Aim of the Study

The aim of this study was therefore to determine the current status of haemosporidian parasites in scavenging chickens (*Gallus gallus domesticus*) and to investigate the level of awareness amongst village poultry farmers in Dukku, Gombe State, Nigeria.

Materials and Methods

Study area

This study was carried out in the Dukku Local Government Area, its headquarters is located in the town of Dukku, Gombe State, Nigeria. The northeasterly line of equal latitude 14°N and longitude 14°E passes through the LGA about 6 km to the southeast of the town of Dukku. The Gongola River flows through the west and north of the LGA. It has an area of 3,815 km^2 and a population of 207,190 at the 2006 census. The climate is characterized by heavy rainfall and high temperatures. The mean temperature varies from 32 - 34^{0}C, Wind S at 8 km/h, 45% Humidity, 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 survey design adopting convenient sampling techniques was used to sample three (3) districts, including Dukku, Gombe Abbah and Hashidu for blood sampling and other data collections. This study involved microscopic examination of thin blood film and buffy coat smear for the presence or absence of intra or extracellular haemoparasites. Village chickens farmers were interviewed to evaluate their awareness on haemoparasites of chickens.

Ethical clearance and study population

Permission for this study was obtained from the Office of Research and Animal Welfare, Ministry of Agriculture and Animal Husbandry, Gombe State. The study population included a total of 370 chickens (*Gallus gallus domesticus*) reared under the extensive management system and were sampled from households that raise various species of village poultry (ducks, turkeys, pigeons, guinea fowls). Chickens ages were considered as growers (3 - 4 months) and adults (over 5 months).

Blood sample collection

Blood samples were aseptically collected from live village chickens (*Gallus gallus domesticus*) via the wing vein (venipuncture) after consent from their owners. Blood samples were obtained during the two seasons, namely the rainy and dry seasons. Thin smears of blood were made on a clean dry slide, allowed to air dry for a few minutes, then fixed in absolute methanol, and then allowed to air dry again, before properly labeling each slide. Slides were carefully packed and arranged in slide boxes for easy transport to the Department of Veterinary Parasitology and Entomology Research Laboratory, University of Maiduguri, Borno State, Nigeria. In the laboratory, thin smears of buffy coat from centrifuged blood samples were also made on a clean dry slide, allowed to air dry for a few minutes, then fixed in absolute methanol, and then allowed to air dry again.

Microscopic detection of haemoparasites

The slides were stained with Giemsa stain (pH 7.2), rinsed with distilled water, and allowed to air dry. Stained thin blood film and buffy coat smears were later viewed under a light microscope for the presence of intracellular or extracellular parasites at low and high magnification oil immersion objective. The haemoparasites schizonts, gametocytes, and trophozoites were examined and classified as previously described by 28 (2000) and 21 (2005).

Data analysis

Data analysis was performed using GraphPad Prism software (GraphPad Inc., San Diego, CA). Prevalence rates which represent the current status of haemoparasites in the study area were calculated as percentages of proportion. The level of awareness of haemoparasites in chickens amongst village poultry farmers were also calculated as percentages of responses to the interview. Chi-squared test was used to compare categorical variables (age, sex and season). Differences were considered significant for *p*-values equal to or less than 0.05.

Results

Table 1 summarized the results of species of avian haemosporidian parasites amongst scavenging chickens (*Gallus gallus domesticus*) in Dukku, Gombe State, Nigeria. The result shows a higher prevalence of single *Plasmodium* infection (15.9%; 95% CI = 12.6% - 20.0%) compared to *Haemoproteus* infection (4.1%, 95% CI = 2.5% - 6.6%), while the prevalence of mixed *Plasmodium* and *Haemoproteus* infection was found to be 1.9%; 95% CI = 0.9% - 3.9%.

Type of Infection	Haemoparasites	No. of chickens infected N = 370	Prevalence (%)	95% CI LL - UL
Single	*Plasmodium* spp.	59	15.9	12.6 - 20.0
	Haemoproteus spp.	15	4.1	2.5 - 6.6
Mixed	*Plasmodium* spp.+ *Haemoproteus* spp.	7	1.9	0.9 - 3.9
Overall		81	21.9	

Table 1: *Species of avian haemosporidian parasites amongst scavenging chickens (Gallus gallus domesticus) in Dukku, Gombe State, Nigeria.*

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

Table 2 summarized the results of the current status of haemoparasites causing avian malaria amongst scavenging chickens (*Gallus gallus domesticus*) in Dukku, Gombe State, Nigeria. Haemosporidian parasites infections were found in 81 chickens with an overall prevalence rate of 21.9% of the total 370 scavenging chickens sampled and examined. The current status of infection in Gombe Abbah (9.2%; 95% CI = 6.7% - 12.6%) was found to be higher followed by Dukku (7.6%; 95% CI = 5.3% - 10.7%) and Hashidu (5.1%, 95% CI = 3.3% - 7.9%) in a descending order of prevalent rate.

Study areas	No. of chickens examined	No. of chickens infected (%)	Prevalence (%)	95% CI LL - UL
Dukku	122	28 (23.0)	7.6	5.3 - 10.7
Gombe Abbah	126	34 (27.0)	9.2	6.7 - 12.6
Hashidu	122	19 (15.6)	5.1	3.3 - 7.9
Overall	370	81 (21.9)	21.9	18.0 - 26.4

Table 2: *Current status of haemoparasites causing avian malaria amongst scavenging chickens (Gallus gallus domesticus) in Dukku, Gombe State, Nigeria.*

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

Table 3 summarized the results of risk factors associated with haemosporidian parasite infections amongst scavenging chickens (*Gallus gallus domesticus*) in Dukku, Gombe State, Nigeria. Considering sex as a risk factor, the prevalence of avian haemosporidian parasites in male (cocks) (14.30%) was found to be relatively higher than in female (hen) (7.6%) chickens, and the association between avian haemosporidian parasites prevalence and sex of chickens was found to be statistically significant (*P*-value = 0.0061; χ^2 = 7.525; OR = 2.100). However, the prevalence of avian haemosporidian parasites was found to be relatively higher in adults (16.5%) compared to grower chickens (5.4%) and the association between prevalence and age group of chickens was also found to be statistically significant ($P < 0.0001$; χ^2 = 17.784; OR = 2.291). The prevalence of avian haemosporidian parasites was found to be higher in the rainy season (18.4%) compared to the dry season (3.5%) of the sampling period; and the association between haemosporidian parasites prevalence and sampling season was also found to be statistically significant ($P < 0.0001$; χ^2 = 46.090; OR = 7.690).

Risk factors	No. of chickens examined	No. of chickens infected (%)	Prevalence (%)	*P*-value	95% CI LL – UL	OR	χ^2	*P*-value
Sex								
Male	190	53 (27.9)	14.3[a]	0.0061	11.1 – 18.3	2.100	7.525	0.0061
Female	180	28 (15.6)	7.6[b]		5.3 – 10.7			
Age (months)								
Adults (> 5)	200	61 (30.5)	16.5[a]	<0.0001	13.1 – 20.6	2.291	17.784	<0.0001
Grower (3 - 4)	170	20 (11.8)	5.4[b]		3.5 – 8.2			
Season								
Rainy	185	68 (36.8)	18.4[a]	<0.0001	14.8 – 22.6	7.690	46.090	<0.0001
Dry	185	13 (7.0)	3.5[b]		2.1 – 5.9			

Table 3: *Haemoparasites causing Avian Malaria and some its associated risk factors amongst Scavenging Chickens (Gallus gallus domesticus) in Dukku, Gombe State, Nigeria.*

[a,b] *Different superscripts indicate significant ($p < 0.05$) difference.*

Key: LL= Lower limit; UL= Upper limit; CI= Confidence Interval; χ^2 = Chi-square; OR = Odd Ratio.

The results on the responses of village poultry farmers on their awareness of the occurrence of haemoparasites in village chickens in the present study area were represented in figure 1. Out of 45 respondents, none 0 (0.0%) of the village poultry farmers reported being aware of the occurrence of haemoparasites in village chickens while all the respondents 45 (100.0%) reported that they were not aware. There was a statistical significant difference ($p < 0.05$) between the village poultry farmers' responses.

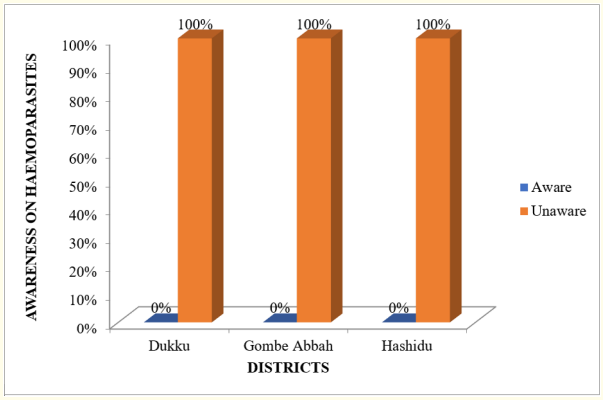

Figure 1: *Graphical representation of Awareness of village poultry farmers on occurrence of haemoparasites in village chickens in Dukku Local Government Area, Gombe State, Nigeria.*

Discussion

In most developing countries, the recent corona virus (covid-19) pandemic has crippled the national economy and exposed the less privileged populace to hunger and poverty. There is need to improve livestock production in order to significantly curtain protein deficiencies among people during the covid-19 lockdown period. Village chicken production system would practically be the promising source of food security and petty cash in Africa to keep up the high animal protein demand and alleviate poverty. Avian malaria caused by haemoparasites can pose a significant threat to the production of village chicken where strict biosecurity and awareness campaigns are not correctly put in place (Figure 2-4).

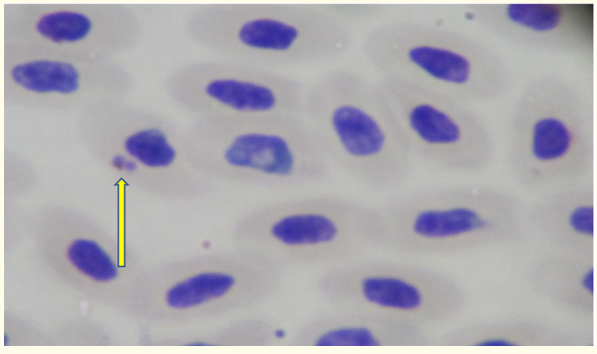

Figure 2: *Plasmodium spp. in blood smear (x1,000). Pigmented gametocytes are present within the cytoplasm of mature erythrocytes (Yellow arrow).*

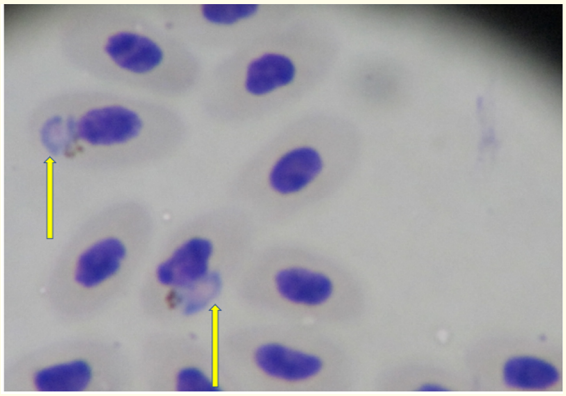

Figure 3: *Haemoproteus in blood smear (x400). Pigmented gametocytes curving around the nucleus of a mature erythrocyte (Yellow arrows).*

Figure 4: *Village chickens scavenging in unhygienic environment in Dukku Local Government Area, Gombe State, Nigeria.*

The results of this study revealed an overall prevalence of 21.9% for haemoparasites in village chickens using microscopy. This findings is higher than the findings of 29 (2004) who recorded 2.4% in Zaria, Kaduna State; 30 (2012) who reported 12.0% in Sokoto as well as 31 (2008) who reported 11.4% in Maiduguri, Borno State respectively, but lower than the 26.4% recorded in Ibadan, Oyo State [32], 46.7% in Owerri, Imo State [20] and 23.2% recorded in Makurdi, Benue State [33]. These variations in the prevalence rates from various studies might be attributed to differences in sample sizes, geographical regions as well as abundance of arthropod vectors and season which may influence vector breeding. In addition, higher prevalence rates of avian malaria parasites in scavenging chickens have been recorded in some parts of Africa such as 43.4% in Ethiopia [34], 79.2% in Kenya [14], 79.1% in Malawi by 35 (2015), 61.9% in Uganda [36], 35.0% in Ghana [32], and 32.0% in Zimbabwe [38]. Similar studies from different parts of the world have also reported higher prevalence of haemoparasites in Asia, prevalences of 69.0%, 34.5%, 54.6%, 45.6% and 42.0% were reported in Punjab, Pakistan, Bangladesh and Philip-

pine respectively [39-42]. 43 (2007) have recorded 34.0% in India and 44 (2013) reported 78.2% in Iraq. These various findings revealed evidence of occurrence of avian haemosporidian in scavenging village chickens from different parts of the world which were all attributed to differences in diagnostic methods used for the avian haemoparasites detections, type of samples investigated, difference in ecological, climatic and other geographic (altitudinal) factors which might influence abundance of vectors and also differences in husbandry and management systems adopted for rearing of village chickens. Other reasons for dissimilarities in prevalence rates might be attributed to variations in genotypic make up and phenotypic appearance, age and sex of the chickens.

In this study, two avian malaria parasites namely *Plasmodium* spp., and *Haemoproteus* spp. were prevalent, which confirms the presence of their suitable vectors in the study area. This finding is consistent with the report of 40 (2014) who explained that *Plasmodium* and *Haemoproteus* species are the most reported avian malaria parasites in scavenging chickens and are prevalent in areas with a suitable ecology and abundance of biting haematophagous arthropods. In this study, mixed infections with *Plasmodium* and *Haemoproteus* species was also found in the infected chicken which tallies with the reports of earlier researchers [14, 26, 33, 35, 36, 41, 42, 45-47]. However, 34 (2017) in Ethiopia and 38 (2002) in Zimbabwe both reported *Plasmodium*, *Leucocytozoon*, *Aegyptianella* and *Trypanosoma* species in scavenging chickens. In this study *Leucocytozoon*, *Aegyptianella* and *Trypanosoma* species were not detected microscopically in all the samples examined. It is important to note that 20 (2016) in Owerri, Nigeria reported the occurrence of *Plasmodium* and *Microfilaria* species in scavenging chickens but their study did not find *Haemoproteus* and *Leucocytozoon* species. The variations in species of haemoparasites encountered in scavenging chickens from various studies may be influenced by the prevalence and abundance of arthropod vectors responsible for transmitting the parasites, methods used for detection, difference in husbandry and management systems in practice, ecological and topographic factors which might influence the abundance of vectors.

In this study, *Plasmodium* species (15.9%) was the most prevalent avian malaria parasites of scavenging chickens and this concurs with the report of 48 (2005) and 31 (2008) who reported that mosquitoes, being vectors of *Plasmodium* species, are endemic in this environment and also reported by other investigators [14,26,34,41,46,49]. It was observed in this study that scavenging chickens are seldom provided with suitable housing or are housed in enclosures without arthropod screening nets; this could expose them to bites by several arthropod vectors including mosquitoes. The finding of this study is in contrast to those of 47 (2015) and 41 (2017) who reported *Haemoproteus* and *Leucocytozoon* species respectively as the most encountered avian haemoparasites in free range chickens in their respective studies. The prevalence rate of *Plasmodium* species in this study is higher than 11.4% previously reported in Maiduguri, Borno State Nigeria [31] and 12.0% reported in Sokoto, Sokoto State Nigeria [30] but lower than 33.3% reported in Owerri, Imo State Nigeria [20]. The reason for variation in the reported prevalence rates of avian malaria parasites in scavenging chickens in some parts of Nigeria might be attributed to the abundance of vectors, variation in ecologic and climatic factors.

The present work revealed the prevalence of single infection of *Haemoproteus* species as 4.1%. This prevalent rate is relatively higher than 0.8%, 0.9% and 2.5% reported by 26 (2014), 14 (2011) and 41 (2017) from Selangor, Ethiopia and Bangladesh respectively. The prevalence of *Haemoproteus* species in village chickens reported in this present study is lower than 23.3% reported by 50 (2013) in Bangladesh; 13.2% by 47 (2015) in Iraq and 50.9% in Maiduguri, Nigeria [27]. This might be attributed to differences in the geographical locations, abundance of arthropod vectors and season of sample collections.

Based on the district sampled, the prevalence of avian malaria parasites in scavenging chickens was found to be relatively higher in Gombe Abbah (9.2%), followed by Dukku (7.6%), while Hashidu (5.1%), had the lowest prevalence of avian malaria parasites. The high prevalence rate recorded in Gombe Abbah may indicate abundance of arthropod vectors in the study area. However, the variations in prevalence rates might be related to differences in vegetation such as availability of stagnant pools of water, swampy or marshy environments and ecological activities which are usually favourable for the breeding and biology of the vectors, coupled with the scavenging behaviour of the chickens may predispose them to the vector borne infection.

The results of this study revealed a higher prevalence in males (14.3%) compared to female (7.6%) chickens which was statistically significant ($p < 0.05$), and this may be attributed to the facts that cocks anatomically have larger combs and wattles, that are richly supplied with blood vessels which may attract blood sucking arthropods that transmit haemoparasites to the host bird. This finding concur with the report of previous works (20; 27; 33) that also reported higher prevalence of avian malaria parasites in males compared to the female village chickens in Nigeria. The findings of this study are also consistent with previous reports in Africa and Asia [14,34,41,49]. It is important to note that other worker have reported higher prevalence of haemoparasites in the hen [41,42,47,51].

The prevalence of avian malaria parasites infection based on age of village chickens in this study has revealed a higher prevalence in adults (ages > 5 months) compared to the growers (ages 3 - 4 months) which was statistically significant ($p < 0.005$). This might be attributed to the behaviour of adult village chickens which roam far long distance to scavenge for food on or near unhygienic environments and travel far in search of mates, and this predisposes them to higher risks of contact with arthropods vectors, while the growers are usually found near their roosting areas. Also, adult chickens have more prominent and developed combs and wattles which are well vascularized and provide predilection sites for arthropods vectors. This finding concurs with the findings of 40 (2014) in Bangladesh and 34 (2017) in Ethiopia who also reported significantly ($p < 0.05$) higher prevalence of haemoparasites infection in adult chickens compared to growers. However, this finding contradicts that of 14 (2011) in Kenya and 42 (2017) in Pakistan who reported a relatively higher prevalence of avian haemoparasites infection in growers compared to adult chickens, but the difference was not statistically significant ($p > 0.05$).

This study has revealed a significantly ($p < 0.0001$) higher prevalence of avian malaria parasites during the rainy season (18.4%), which is the season that provides favourable conditions such as temperature and humidity that allow breeding of most arthropod vectors such as mosquitoes and other biting flies. The prevalence recorded in this study agrees with the findings of 31 (2008) who also reported a high prevalence of avian haemoparasites during the rainy season in Maiduguri, Nigeria, but contrasts the reports of 41 (2017) in hilly areas of Bangladesh, who reported a prevalence of 60.6% in summer, 36.7% in rainy and 23% in winter seasons.

This study revealed that village chicken farmers are unaware of the occurrence of avian haemoparasites in scavenging chickens. This low level of awareness will no doubt create the ignorance of the need for preventive measures against the avian haemoparasitic diseases of poultry. This finding agrees with the findings of 52 (2014), 53 (2016) and 54 (2017) who also reported that low level of awareness and inadequate extension services militate against the successful production of village chickens in rural areas of Nigeria. Moreover, 55 (2005) have shown that extension services are vital factor in the adoption of technologies in management and disease prevention. 56 (2007), also stated that the more participation of farmers in trainings such as workshops and seminars and hence, the higher the acceptance of new technologies.

Conclusion and Recommendation

In Dukku LGA of Gombe State, Nigeria, two avian malaria parasites (*Plasmodium* and *Haemoproteus* species) were discovered to infect scavenging chickens in a single and mixed infection with an overall prevalent rate of 21.9%. *Plasmodium* species was found to be the most prevalent avian malaria parasite infecting scavenging chickens. Generally, the prevalence of avian malaria parasites were found to be significantly higher in adults compared to grower chickens, the prevalence of infection is also significantly higher in males compared to female chickens and significantly higher in rainy season compared to dry season of the study period. Village chicken farmers in the study area were unaware of the occurrence of avian malaria parasites in scavenging chickens. Therefore, it is also recommended that the avian haemosporidian in scavenging chickens and other village poultry species are to be further studied to their species level using molecular detection tools to further understand the epidemiology of the disease in the study area. Village chicken farmers should be educated on the risk factors of avian malaria parasites through regular extension services with emphasis on control and preventive measures. There is also need to educate poultry farmers on the benefit of adequate management and husbandry system, routine veterinary care and elimination of vectors of haemoparasites in the study area.

Conflict of Interests

The authors declare that they have no conflict of interests in publishing this article.

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