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Survey on Avian Malaria Parasites in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

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ABSTRACT

Reports of avian malaria parasites in village chicken in Nigeria generally remain fragmentary and scarce. The study was conducted in Gombe Local Government Area of Gombe State, Nigeria to investigate avian malaria parasites in Village Chickens (*Gallus gallus domesticus*) and to determine the risk factors associated with the prevalence of the haemoparasites. A total of 530 village chickens blood samples were obtained from apparently healthy village chickens' brachial veins using sterile 2mls syringes and 23 gauge needles. Thin blood smear was made from each blood sample, and Giemsa stained and examined for the presence of avian haemoparasites under an electro-microscope. The result indicates 23.8% overall prevalence rate of three species of avian malaria parasites consisting of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* species. *Plasmodium* spp. has the highest prevalent rate of 13.0% followed by *Haemoproteus* spp. (5.1%), mixed *Plasmodium* spp. + *Haemoproteus* spp. (4.9%) infection and *Leucocytozoon* spp. (0.8%). Prevalence of avian malaria parasites was significantly higher in cocks compared to hens ($p < 0.05$), as well as higher in adults compared to growers chickens ($p < 0.05$). This study also showed a higher prevalence of avian malaria parasites during the rainy season compared to the dry season of the study period. It was concluded that haemoparasites of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* species that occur in both single and mixed infections are prevalent among village chickens that are apparently healthy in Gombe Local Government Area of Gombe State, Nigeria.

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1. Introduction

Among the village poultry species, village chickens also known as 'domestic fowl, rural chickens or local chickens' are the most predominant in Africa's rural poultry sector [1]. Village chickens are typically found in nearly every rural household and raised extensively, with little or no particular attention in terms of husbandry and health care [2,3]. They are commonly found to scavenge around households in unhygienic areas, thus being predisposed to disease vectors like parasitic infection [4,5]. Diseases are regarded as one of the most important constraints for the productivity of village chickens in most developing African countries including Nigeria [3]. The effects of parasitism are often severe on birds [6]. Blood parasites have been reported to influence the evolution and ecology of many bird species [5,7], and are distributed across a wide variety of habitats and geographical regions [8].

Blood parasites have a complex life cycle, usually involving a vector that transmits the infection [9-11]. The frequency and distribution of these vectors varies between habitats, primarily depending on the weather [12]. Several researches dealt with the geographic distribution of genetically distinct blood parasites in different regions and ecosystems [13-15].

Taxonomists have described more than 200 species of avian haemosporidians of hundreds of bird species studied worldwide and categorized them into four distinct genera, *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Hepatocystis* [16,17]. In chickens, the most endemic haemoparasites are *Plasmodium, Haemoproteus* and *Leucocytozoon* [18].

In birds, haemosporidian species can be detected and identified using the method of microscopy that has been considered the "gold standard" diagnostic tool for haemoparasites [9,19], and by amplifying and sequencing DNA [8].

In general, few researches have been documented into the prevalence of avian haemoparasites in poultry species in certain parts of Nigeria [20,21] and other developing countries [22,23]. The most recent research focused on the identification of avian haemoparasites in wild birds [18,24,25]. Studies of the genera of haemoparasites infecting village chicken in Nigeria, particularly in the northeastern region, remain fragmentary and scarce, hence the need to conduct the present study. Therefore, the objective of the study is to determine the genera of haemoparasites that causes avian malaria in village chickens in Gombe Local Government Area of Gombe State, Nigeria.

2. Materials and Methods

Study Area

This study was carried out in Gombe Local Government Area, which is the capital and largest city of Gombe State located in Northeastern Nigeria (Figure 1). Gombe state is located between latitude 9^0 30' and 12^0 3' N and longitude 8^0 45' and 11^0 45' E [26]. The total poultry population in Gombe State is approximately 508, 305 comprising 462,000 backyard poultry and 46,305 exotic poultry [27]. Village poultry farmers' households and live birds markets within the study area were visited for blood sample and other data collection.

Sampling Period

Blood sampling was carried out from apparently healthy during the study period from the month of February, 2019 and January, 2020, within two (2) seasons *viz:* the rainy season (April - September) and dry season (October - February). All study locations were visited for blood samples collection on alternate periods within these study periods.

Study and Target Population

The study population of the present study was village chickens of both sexes and two age groups. Sex of birds, was determined based on their morphology breeding status and plumage. Ages of birds were determined using the maturity of the beak and information from poultry farmers. The age was categorized into adult (above 5 months old) and growers (between 3 - 4 months old). Source of birds during the period of blood sampling were noted (farmer household or live birds markets).

Ethical approval

Ethical approval for the present study was duly obtained from and approved by the Institutional Animal ethics and Research committee of the Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Borno State, Nigeria. After obtaining the consent of poultry farmers and sellers to sample their chickens, the village chickens were caught and caged to rest overnight and prepared for specimen and data collection very early in the morning. Selected village chickens were gently grabbed by the shanks and manually restrained with caution not to allow the chicken go through neither unnecessary struggle nor stress.
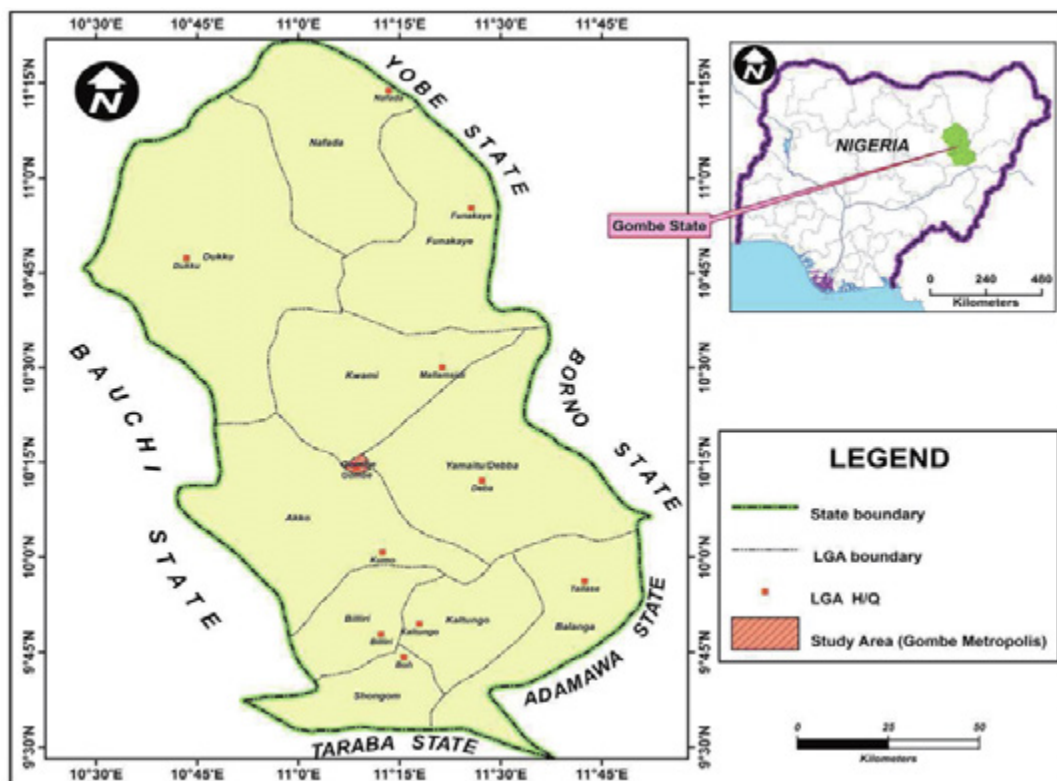
Figure 1. Map of Gombe State showing the study area

Sampling method for birds

Non-probability convenience sampling method was employed. Approximately 3-4 ml of blood samples were obtained aseptically directly from the brachial vein (wing vein) using a 5 ml sterile syringe and 23 gauge needle from each sampled chicken. Each blood sample was immediately dispensed into a sample bottle containing anticoagulant EDTA. All samples were correctly labeled and transported in ice pack container to the Department of Veterinary Parasitology and Entomology Research Laboratory, University of Maiduguri

Identification of Haemoparasites, Parasitological Examination and Detection of Haemoparasites

Thin blood and buffy coats smears of each blood sample were made on two separate slides and left to air dry for a few minutes, then labeled appropriately. The slides were fixed with methanol for five (5) minutes and allowed to air dry, packaged and then stained with diluted 10% Giemsa stain as defined by Zajac and Conboy [28]. The slides were later examined using a microscope at low magnification (x40) and at high magnification (x100) under oil immersion for 10-15 min for the presence of intracellular blood parasites gametocytes as previously described by Valkiūnas [9]. Parasite identification was based on the morphology, height and pigmentation of the endoerythrocytic parasites and photographs compared to the species already described by Taylor *et al.* [29].

Data Analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Inc., San Diego, CA). The prevalence rate was calculated as the ratio of the number of hosts infected by one or more parasite species to the total number of hosts examined as described by Bush *et al.* [30]. Chi-square test / Fisher exact tests were used to compare the proportions obtained for the presence or absence of avian haemoparasites infection and the association between independent variables such as study location, age, sex and season. Differences were considered significant at $p < 0.05$ [31].

3. Results

Out of 530 village chickens blood samples examined, 126 chickens were found positive for one or more avian haemoparasites. Hence, the overall prevalence of avian malaria parasites was 23.8% (95% confidence interval was 20.3% - 27.6%). The results also showed that the prevalence rate of avian haemoparasites was higher in village chickens sampled from poultry farmers'

households (14.9%) compared to those sampled from live birds markets (8.9%) within the study area. Although, the difference in the prevalence rates was not statistically significant (*p*-value = 0.1394; χ^2 = 2.185) as shown in Table 1.

Table 2 presents the results of prevalence of various species of avian malaria parasites in village chickens in the study area. Three (3) different species of avian malaria parasites, namely *Plasmodium* spp.*, Haemoproteus* spp. and *Leucocytozoon* spp., were found in single or mixed infections and with different prevalence in infected chickens. The prevalent rate was highest for *Plasmodium* spp. (13.0%; 95% Confidence Interval = 10.4% - 16.2%) followed by *Haemoproteus* spp. (5.1%; 95% Confidence Interval = 3.5% - 7.3%), mixed *Plasmodium* spp. + *Haemoproteus* spp. infection (4.9%; 95% Confidence Interval = 3.4% - 7.1%) and lowest for *Leucocytozoon* spp. (0.8%; 95% Confidence Interval = 0.3% - 1.9%).

The prevalence of different species of avian malaria parasite based on the study location was shown in Table 3. The prevalence of *Plasmodium* spp. in sampled village chickens from household of poultry farmers (7.7%) was higher than in sampled chickens from live bird markets (5.3%), the difference in their prevalence rates was not statistically significant (*p*-value = 0.7070). *Haemoproteus* spp. prevalence in sampled village chickens from the household of poultry farmers (2.1%) was lower than in sampled chickens from live bird markets (3.0%), difference in the prevalence rate was not statistically significant (*p*-value = 0.1316). Moreover, the prevalence of *Leucocytozoon* spp. in village chickens sampled from the household of poultry farmers (0.6%) was found to be higher than in chickens sampled from live bird markets (0.2%), the difference in the prevalence rate was also not statistically significant (*p*-value = 0.6366). The prevalence of mixed *Plasmodium* spp. and *Haemoproteus* spp. infection in village chickens sampled from the household of poultry farmers (3.0%) was higher than in chickens sampled from live bird markets (1.9%), and difference in the prevalence rate was not statistically significant (*p*-value = 0.7507).

The association of avian malaria parasites species with sex of village chickens in the study area were summarized in Table 4. The findings of this study revealed significantly (*p*-value = 0.0003) higher prevalence of *Plasmodium* spp. in male (9.6%) compared to the females (3.4%) chickens. The prevalence of *Haemoproteus* spp. was also higher in male (4.0%) compared to female (1.1%) chickens, and the association was statistically significant (*p*-value = 0.0092). Moreover, the association between sexes of chickens

and the prevalence of *Leucocytozoon* spp. revealed no significant statistical (*p*-value = 0.1260) difference even though the parasite was only detected in the male (0.8%) and none in the female (0.0%) chickens. However, the association between the prevalence of mixed *Plasmodium* spp. and *Haemoproteus* spp. infection and sexes of chickens revealed significantly (*p*-value = 0.0009) higher prevalence in the male (4.2%) compared to female (0.8%) chickens.

Table 5 summarized the results of age - specific prevalence of avian malaria parasites infections of village chickens in the study area. The results revealed significantly (*p*-value = 0.0050) higher prevalence of *Plasmodium* spp.in adult (10.2%) compared to grower (2.8%) chickens. The prevalence of *Haemoproteus* spp. was also found to be higher in adult (4.3%) compared to grower (0.8%) chickens, and the association was statistically significant (*p*-value = 0.0132). However, the association between the prevalence of *Leucocytozoon* spp. and age group of chickens revealed no significant statistical (*p*-value = 0.3025) difference even though the parasite was only detected in the adult (0.8%) chickens but none in the grower (0.0%). Moreover, the association between the prevalence of mixed *Plasmodium* spp. and *Haemoproteus* spp. infection and age group of chickens revealed significantly (*p*-value = 0.0035) higher prevalence in the adult (4.3%) compared to grower (0.6%) chickens.

Table 6 summarizes the results of the association between the prevalence of avian malaria parasites infection in village chickens and the season of sample collection in the study area. The results revealed higher prevalence of *Plasmodium* spp. in the rainy (10.0%) compared to dry season (3.0%), and the association was statistically (*p*-value < 0.0001) significant. The findings of this study also showed significantly higher (*p*-value = 0.0288) prevalence of *Haemoproteus* spp. in the rainy (3.6%) compared to dry (1.5%) season. In addition, the results of this study also revealed 0.8% prevalent rate of *Leucocytozoon* spp. during the rainy season, whereas *Leucocytozoon* spp. was not detected in the blood samples collected and examined during the dry (0.0%) season, the association between the prevalence and season was considered not statistically significant (*p*-value = 0.0572). The association between the prevalence of mixed *Plasmodium* spp. and *Haemoproteus* spp. infection and season revealed significantly (*p*-value = 0.0010) higher prevalence in the rainy (4.0%) compared to dry (0.9%) season.

Table 1. Overall Prevalence of Avian Malaria Parasites in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

Sampling Location	Number Examined	Number (%) Infected (95% CI)	Prevalence (%)	*p*-value	χ^2	Relative Risk
Live Birds' Markets	230	47 (20.4) (15.7 - 26.1)	8.9[a]	0.1394	2.185	1.080
Poultry Farmers' Households	300	79 (26.3) (21.7 - 31.6)	14.9[a]			
	530	126 (23.8) (20.3 - 27.6)	23.8			

Table 2. Prevalence of Avian Malaria Parasites Encountered in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

Avian Malaria Parasites Encountered	Number Infected (N = 530)	Prevalence (%)	95% Confidence Interval Lower Limit - Upper Limit
Plasmodium spp.	69	13.0	10.4 - 16.2
Haemoproteus spp.	27	5.1	3.5 - 7.3
Leucocytozoon spp.	4	0.8	0.3 - 1.9
Plasmodium spp. + *Haemoproteus* spp.	26	4.9	3.4 - 7.1
Total	126	23.8	20.3 - 27.6

N = Number of samples examined

Table 3. Study location - Specific Prevalence of Avian Malaria Parasites in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

Avian Malaria Parasites	Study Location	Number Examined	Number (%) Infected (95% CI)	Prevalence (%)	*p* - value	χ^2	Relative Risk
Plasmodium spp.	Live birds Markets	230	28 (12.2) (8.6 - 17.0)	5.3[a]	0.7070	0.1413	1.017
	Poultry Farmers households	300	41 (13.8) (10.2 - 18.0)	7.7[a]			
Haemoproteus spp.	Live birds Markets	230	16 (7.0) (4.3 - 11.0)	3.0[a]	0.1316	2.274	0.9658
	Poultry Farmers households	300	11 (3.7) (2.1 - 6.5)	2.1[a]			
Leucocytozoon spp.	Live birds Markets	230	1 (0.4) (0.1 - 2.4)	0.2[a]	0.6366	-	1.006
	Poultry Farmers households	300	3 (1.0) (0.3 - 2.9)	0.6[a]			
Plasmodium spp. + *Haemoproteus* spp.	Live birds Markets	230	10 (4.4) (2.4 - 7.8)	1.9[a]	0.7507	0.1010	1.010
	Poultry Farmers households	300	16 (5.3) (3.3 - 8.5)	3.0[a]			

[a,b] Different superscripts indicate significant (p < 0.05) difference in study location-specific prevalence; χ^2 = Chi-square

CI = Confidence Interval

Table 4. Sex - Specific Prevalence of Avian Malaria Parasites in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

Avian Malaria Parasites	Sex	Number Examined	Number (%) Infected (95% CI)	Prevalence (%)	p - value	χ^2	Relative Risk
Plasmodium spp.	Male (Cock)	280	51 (18.2) (14.1 - 23.2)	9.6[a]			
	Female (Hen)	250	18 (7.2) (4.6 - 11.1)	3.4[b]	0.0003	13.193	0.8813
Haemoproteus spp.	Male (Cock)	280	21 (7.5) (5.0 - 11.2)	4.0[a]			
	Female (Hen)	250	6 (2.4) (1.1 - 5.1)	1.1[b]	0.0092	-	0.9477
Leucocytozoon spp.	Male (Cock)	280	4 (1.4) (0.6 - 3.6)	0.8[a]			
	Female (Hen)	250	0 (0.0) (0.0 - 1.5)	0.0[a]	0.1260	-	0.9857
Plasmodium spp. + *Haemoproteus* spp.	Male (Cock)	280	22 (7.9) (5.3 - 11.6)	4.2[a]			
	Female (Hen)	250	4 (1.6) (0.6 - 4.0)	0.8[b]	0.0009	-	0.9364

[a,b] Different superscripts indicate significant (p < 0.05) difference in sex-specific prevalence; χ^2 = Chi-square; CI = Confidence Interval

Table 5. Age - Specific Prevalence of Avian Malaria Parasites in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

Avian Malaria Parasites	Age Group (months)	Number Examined	Number (%) Infected (95% CI)	Prevalence (%)	p - value	χ^2	Relative Risk
Plasmodium spp.	Adult (> 5)	330	54 (16.4) (12.8 - 20.7)	10.2[a]			
	Grower (3 - 4)	200	15 (7.5) (4.6 - 12.0)	2.8[b]	0.0050	7.875	0.9042
Haemoproteus spp.	Adult (> 5)	330	23 (7.0) (4.7 - 10.2)	4.3[a]			
	Grower (3 - 4)	200	4 (2.0) (0.8 - 5.0)	0.8[b]	0.0132	-	0.9493
Leucocytozoon spp.	Adult (> 5)	330	4 (1.2) (0.5 - 3.1)	0.8[a]			
	Grower (3 - 4)	200	0 (0.0) (0.0 - 1.9)	0.0[a]	0.3025	-	0.9879
Plasmodium spp. + *Haemoproteus* spp.	Adult (> 5)	330	23 (7.0) (4.7 - 10.2)	4.3[a]			
	Grower (3 - 4)	200	3 (1.5) (0.0 - 1.9)	0.6[b]	0.0035	-	0.9445
Total		530	26 (4.9) (3.4 - 7.1)				

[a,b] Different superscripts indicate significant (p < 0.05) difference in age-specific prevalence; χ^2 = Chi-square; CI = Confidence Interval

Table 6. Season - Specific Prevalence of Avian Malaria Parasites in Village Chickens (*Gallus gallus domesticus*) in Gombe Local Government Area, Gombe State, Nigeria

Avian Malaria Parasites	Season	Number chickens examined	Number chickens infected	Prevalence (%)	χ^2	P - value	Relative Risk
Plasmodium spp.	Dry	270	16 (5.9) (3.7 - 9.4)	3.0[a]	23.192	< 0.0001	1.182
	Rainy	260	53 (20.4) (15.9 - 25.7)	10.0[b]			
Haemoproteus spp.	Dry	270	8 (3.0) (1.5 - 5.7)	1.5[a]	-	0.0288	1.047
	Rainy	260	19 (7.3) (4.7 - 11.1)	3.6[b]			
Leucocytozoon spp.	Dry	270	0 (0.0) (0.0 - 1.4)	0.0[a]	-	0.0572	0.9846
	Rainy	260	4 (1.5) (0.6 - 3.9)	0.8[a]			
Plasmodium + *Haemoproteus* spp.	Dry	270	5 (1.9) (0.8 - 4.3)	0.9[a]	-	0.0010	1.068
	Rainy	260	21 (8.1) (5.4 - 12.0)	4.0[b]			

[a,b] Different superscripts indicate significant (p < 0.05) difference in season-specific prevalence; χ^2 = Chi-square; CI = Confidence Interval
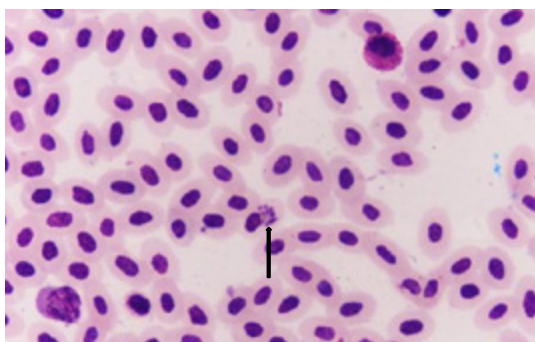

Figure 2. Chicken blood smear showing RBC infected with *Haemoproteus* species. Pigmented gametocytes curving around the nucleus of a mature erythrocyte (arrow)
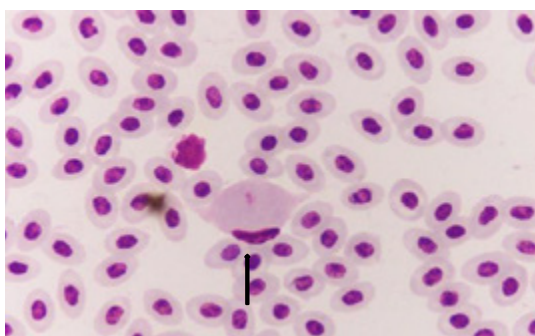

Figure 4. Chicken blood smear showing RBC infected with *Plasmodium* species. Pigmented gametocytes are present within the cytoplasm of mature erythrocytes (arrow)

Figure 3. A photomicrograph showing *Leucocytozoon* infection in blood smear (100x). Large basophilic organisms seen distending the avian WBCs (note malformed nucleus of host cell) (arrow)
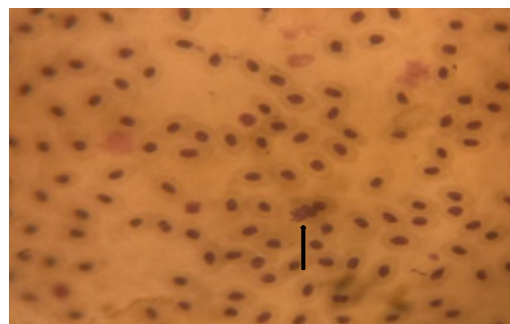

Figure 5. Chicken blood smear showing RBC infected with *Plasmodium* species. Pigmented gametocytes are present within the cytoplasm of mature erythrocytes (arrow)
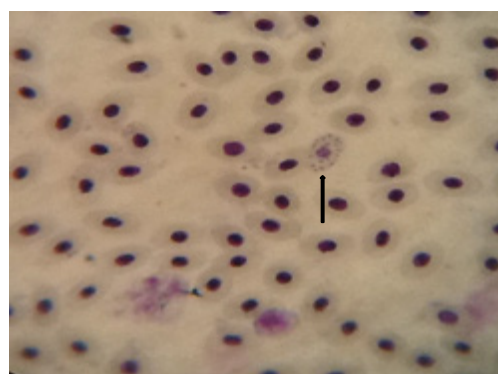
4. Discussion

The detection of haemosporidians that causes avian malaria in 23.8% of village chickens examined in this study indicated the presence of the parasites and abundance of arthropod vectors capable of transmitting them to scavenging village chicken flocks in the study area. From the result of the present study, the prevalence of avian malaria parasites was higher in village chickens sampled at farmers' households (14.9%) compared to those sampled from the live birds markets (8.9%). Although, the association between the prevalence and sample location was not statistically (*p*-value = 0.1394) significant. This finding indicated equal chances of getting infection from the two sample locations. However, the major reasons that might be responsible for higher prevalent rates from households may be associated with scavenging nature of the chickens in unhygienic environment, inadequate husbandry and management systems which usually predispose the chickens to high chance of bites from several blood sucking arthropods capable of transmitting haemoparasites including avian malaria, compared to movement restriction of chickens in live birds markets. These findings buttress previous reports of Igbokwe *et al*. [20] and Ogbaje *et al*. [32] who have also reported high prevalent rate of avian haemoparasites in free range chickens compared to chickens sampled from live birds' markets or chickens in hygienic environment. Moreover, the detection of avian malaria parasites in apparently healthy village chickens from live birds markets may not be unexpected, because the chickens are in most instances sourced directly from poultry farmers households and when they gets to the live birds markets, there are usually no discriminations of health status or screening for diseases before mixing chickens from different sources.

The present study discovered three species of avian malaria parasites, namely *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp., infecting village chickens in the study area. This finding indicates the presence of sufficient blood sucking arthropod vectors capable of transmitting these parasites to village chickens and probably to other domesticated poultry species in the study area. The findings from this research agree with those of Walther *et al*. [33] and Nourani *et al*. [34] who identified these three avian haemosporid parasites as the most prevalent haemoparasites in birds. The present study has also found mixed *Plasmodium* spp. and *Haemoproteus* spp. infections in infected chickens. Discoveries from this study supported previous findings from Kenya [23], Malaysia [35], Iraq [36], Malawi [37], Bangladesh [38], Pakistan [39] and Benue, Nigeria [32]; where these three species of avian malaria parasites were also reported in various similar studies in free range chickens. In some parts of Nigeria, only *Plasmodium* spp. were identified from Maiduguri and Sokoto by Igbokwe *et al*. [20] and Usmana *et al*. [40], respectively. In another study, Opara *et al*. [41] reported only *Leucocytozoon* while Opara *et al*. [42] reported *Plasmodium* and *Microfilaria* in Imo State in scavenging chickens. Still from Nigeria, Karamba *et al*. [43] and Lawal *et al*. [44] published on *Plasmodium* spp. and *Haemoproteus* spp. from Kano and Maiduguri respectively. While from other parts of Africa, *Plasmodium*, *Leucocytozoon*, *Aegyptianella* sand *Trypanosoma* species have been identified in village chickens from Zimbabwe [45] and Ethiopia [46]. In addition, Poulsen *et al*. [47] and Njunga [48] have reported *Plasmodium* and *Aegyptianella* species in scavenging chickens from Ghana and Malawi respectively. Gimba *et al*. [35] found *Plasmodium, Haemoproteus, Leucocytozoon, Microfilaria* and *Trypanosoma* species in village chickens in Malaysia, while Hasan *et al*. [49] reported *Plasmodium* and *Leucocytozoon* species in village chickens in Bangladesh. The variation in the species of haemoparasites discovered in village chickens from different studies could be influenced by the topographical factors, availability and abundance of arthropod vectors responsible for the transmission of the parasites, as well as difference in husbandry and management systems of chickens. In addition, other suggestive reasons that may influence the pervasiveness of avian haemoparasites infection may include season of sampling, sampling effort, differences in habitat and climate [19,50].

Amongst the three species of haemoparasites detected in the present study, *Plasmodium* species (13.0%) was the most prevalence followed by *Haemoproteus* (5.1%), mixed *Plasmodium* spp. and *Haemoproteus* spp. (4.9%) while the prevalence of *Leucocytozoon* spp. (0.8%) was the least in the infected village chickens. The finding of *Plasmodium* species as the most prevalent haemoparasites in this study may be correlated with the abundance of mosquitoes in the study area being the common vectors of *Plasmodium* species. This finding concurs with previous reports which have discovered that several families of blood sucking arthropods vectors including mosquitoes which are capable of transmitting avian malaria are highly prevalent in the Northeastern Nigeria including the present study area [20,51]. Zhang *et al*. [18], Sabuni *et al*. [23], Nath and Bhuiyan [38], Etisa *et al*. [46], Hasan *et al*. [49], Sadiq *et al*. [52] and Shadan [53], who described *Plasmodium* species in their different study areas as the most encountered avian haemoparasites in scavenging village chickens, supported

by the findings of the current study. The findings of the present study contrast those of Hasson [36] and Permin *et al.* [45] who reported *Haemoproteus* species, Nath and Bhuiyan [38] who reported *Leucocytozoon* species and Hasson [36] who reported mixed *Plasmodium* and *Heamoproteus* species infection as the most common avian haemoparasites in village chickens in their various studies. Variation in the sampling season, abundance of vectors, method of diagnosis, efforts of sampling, geographical factors, variation in ecological and climatic factors as well as variation in the management and husbandry systems used in the rearing of village chickens may be attributed to the explanation for disparity in the recorded prevalent rates and incidence of avian haemoparasites in village chickens.

In the current study, the prevalence of *Plasmodium* (13.0%) species found in village chickens is greater than 11.4% recorded in Maiduguri, Borno State Northeastern Nigeria [20] and 12.0% in Sokoto, Sokoto State Northwestern Nigeria [40], but lower than 33.3% in Owerri, Imo State Southeastern Nigeria [42] and 32.0% in Ibadan, Oyo State Southwestern Nigeria [52]. Some parts of Africa have recorded varying prevalence rates of *Plasmodium* species that are higher than the results of this current study. In Ghana, Poulsen *et al.* [47] reported a prevalence rate of 27%, 18.2% have been reported in Ethiopia [46], 15% in Malawi [48], 14.9% in Zimbabwe [45], Mbuthia *et al.* [54] and Sabuni [22] reported 29.8% in Kenya.

The finding of the present study also showed a prevalence rate of 5.1% of *Haemoproteus* species in village chickens. This finding is higher than 1.3%, 0.8%, 0.9% and 2.5% reported by Sadiq *et al.* [52], Gimba *et al.* [35], Sabuni *et al.* [23] and Nath and Bhuiyan [38] from Ibadan, Malaysia, Ethiopia and Bangladesh respectively. In the present study, the prevalence of *Haemoproteus* species recorded is lower than 23.3% reported in Bangladesh [55] and 13.2% in Iraq [36]. The disparity in geographic distribution, management systems, and vector abundance may be some of the reason for these differences in prevalence rates.

The result of the present study revealed 4.9% prevalent rate of mixed *Plasmodium* spp. and *Haemoproteus* spp. infection. This finding is higher than 3.5% and lower than 47.4% reported by Sabuni *et al.* [23] and Hasson [36] respectively.

The result of the present study showed a prevalence of 0.8% of *Leucocytozoon* spp. in village chickens in the study area. This finding is lower than 20.0% reported in Ibadan [52] and 8.9% in Owerri [41]. Compared to other haemoparasites, the low prevalence of *Leucocytozoon* spp. in village chickens found in the present study may be due to the sparse abundance of arthropod vectors capable of transmitting the parasites in the study area. In some parts of Africa, higher prevalence of the *Leucocytozoon* species has previously been reported from village chickens, such as 31.6% in Kenya [54], Sabuni *et al.* [23] reported 31.6% in Ethiopia, Permin *et al.* [45] reported 4.3% from Zimbabwe, while Sabuni [22] and Sehgal *et al.* [56] have also reported 52.1% and 31.0% from Kenya and Uganda respectively. Moreover, 14.5% prevalence of *Leucocytozoon* spp. has been reported from Bangladesh [38], 6.8% reported from Garut [57] and 24.4% from Pakistan [39]. These findings specify the occurrence of *Leucocytozoon* species and suitable vectors for the transmission of this haemoparasite amongst village chickens in these parts of the world. However, the reasons for variation in the prevalent rates from various studies might be attributed to variation in ecologic and climatic factors, as well as dissimilarity in the management and husbandry systems in the rearing of village chickens.

The result of the present study also considered the association between prevalence of avian malaria parasites and sex of village chickens in the study area. This result of this study revealed statistical significant (*p*-value = 0.0003) association between prevalence of *Plasmodium* spp. and sex of chickens in the study area. The prevalence was found to be higher in male (9.6%) compared to the female (3.4%) chickens. This finding might be attributed to the facts that the male (cocks) chickens customarily have larger comb and wattle compared to the females (hen). The fact that the comb is richly vascularized and easily accessed by blood sucking arthropods including the mosquitoes, this might enhance ease transmission of the parasite during blood meal by infected arthropods. This finding agrees with that of Valkiūnas [9], Valkiūnas *et al.* [58], Opara *et al.* [42] and Hasan *et al.* [49] who have reported predominant prevalence of avian *Plasmodium* species in cocks than in the hens of scavenging chickens. Moreover, in an experimental infection involving exposure of both sexes of chickens to *Plasmodium* infected arthropods, the cocks were reported to be more infected with avian malaria, showing all evidence of the disease and corresponding clinical signs [58,59]. Several researches have also shown high prevalence of avian malaria due to *Plasmodium* species in male birds compared to their female counterparts [19,59, 60-63]. However, the result of this present study is inconsistent with the findings of Etisa *et al.* [46] who have reported higher prevalent of *Plasmodium* infections in hens compared to the cocks, and Igbokwe *et al.* [20] who have reported equal prevalent rates amongst both sexes of village chickens.

The result of the present study revealed higher

prevalence of *Haemoproteus* species in male (4.0%) compared to the female (1.1%) chickens; and association between prevalence and sex was statistically significant (*p*-value = 0.0092) at 95% confidence interval. The findings of this present study agrees with Islam *et al.* [55] who have also reported higher prevalence of *Haemoproteus* species cocks compared to the hens. The finding of this study was not consistence with Sabuni *et al.* [23] who reported high prevalence of *Haemoproteus* species in hens (4.2%) compared to the cocks (2.8%).

The prevalence of mixed *Plasmodium* and *Haemoproteus* species infection in village chickens from the present study also revealed higher prevalent rate in male (4.2%) compared to the female (0.8%) chickens, and the association between the prevalence rate and sex was statistically significant (*p*-value = 0.0009) at 95% confidence interval. To the best of our knowledge, this is the first reported prevalence of mixed *Plasmodium* + *Haemoproteus* species infection in village chickens in Nigeria, considering the sexes as risk factor. However, high prevalence of mixed infection in cocks compared to hens might also be attributed to more prominent blood sucking sites on the cocks compared to hens, even though, the hens also have comb and wattle, but are smaller compared to the cocks. The blood sucking arthropod vectors usually prefers more accessible and less feathered parts of the host birds during blood meal [64].

The result of the present study only detected *Leucocytozoon* species in male (0.8%) and the parasite was not detected in female (0.0%) chickens. The finding of the present study agrees with Sabuni *et al.* [23] who have reported higher prevalence rate of *Leucocytozoon* species in cocks (54.2%) compared to the hens (50.0%) village chickens. However, the result of the present study is inconsistent with Etisa *et al.* [46] and Hasan *et al.* [49] who have reported higher prevalence of *Leucocytozoon* species in hens compared to the cocks of village chickens. The susceptibility of village chickens to *Leucocytozoonosis* is determined by the abundance of the vector responsible for the transmission of the parasite among host [46]. Few studies that have reported the prevalence of *Leucocytozoon* species in village chickens did not revealed it's the association between its prevalence and sex of chickens [36,38,39].

The findings of the present study revealed higher prevalence of *Plasmodium* species in adult (10.2%) compared to the grower (2.8%) chickens; the association between the prevalent rates and age group was statistically significant (*p*-value = 0.0050) at 95% confidence interval. This finding concurs with Etisa *et al.* [46] who have also reported high prevalence of *Plasmodium* species in

adult (20.1%) compared to the grower (10.1%) village chickens. Moreover, *Plasmodium* species have been reported to be highly pathogenic in adult chickens compare to younger ones, and mortality rates ranges from 30 - 80% [65]. However, Hasan *et al.* [49] have reported high prevalence of *Plasmodium* species amongst younger (2.9%) compared to the adult (1.2%) domesticated chickens. However, Sabuni *et al.* [23] reported that adult and grower birds shares equal chances of getting infection where exposed to *Plasmodium* infection where suitable vectors are abundant. While in Zimbabwe, Permin *et al.* [45] reported that the differences in prevalence of *Plasmodium* species were not significantly different between the bird's ages (young and adult). The present study represents the first reports on comparison of occurrences of *Plasmodium* species between village chickens' ages in Nigeria. The few reports from some parts of Africa revealed that differences in prevalence of *Plasmodium* species among ages of chickens is most likely connected to abundance of arthropod vectors and variations in exposure of host to infected vectors [45].

The prevalence of *Haemoproteus* species in village chickens from the present study revealed higher prevalent rate in adult (4.3%) compared to the grower (0.8%) chickens; the association between prevalence and age group was statistically significant (*p*-value = 0.0132) at 95% confidence interval. This finding is consistent with that of Islam *et al.* [55] who have reported high prevalence of *Haemoproteus* species in adult compare to the grower chickens. However, Sabuni *et al.* [23] have reported 4.2% prevalent rate of *Haemoproteus* specie in growers and did not detect the parasite in adult village chicken. Moreover, the finding of the present study coincides with Samani *et al.* [66] and Momin *et al.* [67] who in a similar study reported higher prevalence of *Haemoproteus* species in adult compared to young pigeons. The present study also represents the first reports on comparison of occurrences of *Haemoproteus* species between village chickens based on age groups in Nigeria.

The prevalence of mixed *Plasmodium* and *Haemoproteus* species infection in village chickens from the present study also revealed higher prevalent rate in adult (4.3%) compared to the grower (0.6%) chickens; and the association between the prevalent rates and age group was also statistically significant (*p*-value = 0.0035) at 95% confidence interval. The finding of the present study could not be thoroughly compared and discussed due to paucity of literature on the prevalence of mixed *Plasmodium* and *Haemoproteus* species infection in village chickens according to the age group. The present study also represents the first report of mixed *Plasmodium*

and *Haemoproteus* species infection in village chickens in Nigeria.

The findings of the present study revealed higher prevalence of *Leucocytozoon* species in adult (0.8%), but the parasite was not detected in grower (0.0%) village chickens. The finding of the present study is consistent with that of Sabuni *et al.* [23] who reported that *Leucocytozoon* species showed an increase in prevalence rate with increase in age of chicken. The finding of the present study did not concur with those of Etisa *et al.* [46] and Hasan *et al.* [49] who have reported higher prevalence of *Leucocytozoon* species in grower compared to the adult village chickens. However, Sehgal *et al.* [68] in a similar study reported that young birds are more susceptible to *Leucocytozoon* species than adults, and the most serious mortality generally occurs within the first few weeks of hatching.

The prevalence of avian malaria parasites according to season revealed significantly higher prevalence of *Plasmodium* species during the rainy (10.0%) compared to dry (3.0%) season. This finding agrees with Igbokwe *et al.* [20] and Okanga *et al.* [69] who have also reported high prevalence of *Plasmodium* species during the raining season compared to other seasons. The prevalence of *Haemoproteus* specie recorded in the present study was found to be significantly higher during the rainy season (3.6%) compared to dry (1.5%) season. The detection of this parasite might be connected with the rainy season considered as the favourable breeding season for several species of flies and other arthropod, which are capable of transmitting *Haemoproteus* specie to susceptible chickens. This agrees with the findings of Islam *et al.* [55] and Smith and Ramey [70] who have also reported high prevalence of *Haemoproteus* species in domesticated poultry species and waterfowls during the raining season. According to Adriano and Cordeiro [71], *Haemoproteus* species is reported to be transmitted by blood sucking insects including mosquitoes, biting midges (*Culicoides*), louse flies (*Hippoboscidae*) and tabanid flies (*Tabanidae*) whose population usually increases in the rainy season.

The prevalence of mixed *Plasmodium* and *Haemoproteus* species infection was also found to be higher during the rainy season (4.0%) compared to dry (0.9%) season. This finding is consistent with Smith and Ramey [70] who have reported high prevalence of mixed *Plasmodium* and *Haemoproteus* species infection in waterfowls during the raining season.

The prevalence of *Leucocytozoon* specie was only recorded during the rainy season in the present study, but the parasite was not detected in village chickens during the dry season in the study areas. Seasonal factors tend to have contrasting effects upon different vector species; seasonal variation in the prevalence of vector-borne diseases is well documented [69,72], and the finding of the present study are in accordance with findings from other studies of avian haemoparasites [73-76].

5. Conclusions

In conclusion, the present study revealed an overall prevalence of 23.8% for three (3) species of avian malaria parasites among village chickens in the study area, which included *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* species. *Plasmodium* species which have been reported worldwide as the cause of avian malaria in birds was found to be the most prevalent haemoparasites in village chickens in the study area. Prevalence of the three avian malaria parasites were significantly higher in cocks compared to hens, as well as in adults compared to the growers and their prevalence rates was also found to be higher in the rainy season compared to the dry season of the study period.

6. Recommendation

From this present study it was recommended that further researches involving constant surveillances and molecular characterization should be conducted to unveil the true species of the avian haemoparasites infecting village chickens in Nigeria as well as investigating the possible vectors transmitting these haemoparasites amongst the village chickens so as to design adequate biosecurity and control measures. It is also recommended that similar researches should be conducted to determine the prevalence of avian haemoparasites in other village poultry species, and to further understand the epidemiology of avian malaria in village chickens in the study area.

Acknowledgments

Conflict of Interest

The authors declare that they have no competing interests.

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