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Research Article

Natural Infections of Avian Haemosporidian Parasites among Free-Range Chickens (*Gallus gallus domesticus*) in Yamaltu Deba, Gombe State, Nigeria

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Abstract

Avian malaria caused by haemosporidian parasites belonging to *Plasmodium, Haemoproteus* and *Leucocytozoon* species transmitted by arthropod vectors is considered a global problem for avian species. Despite this concern, there are limited studies on avian haemosporidian parasites in free-range chickens in Nigeria and its sub regions. The aim of this study is to investigate the occurrence of natural infection of avian haemosporidian parasites among free-range chickens in Yamaltu Deba, Gombe State, Nigeria. To achieve this goal, blood samples were obtained from 400 apparently healthy chickens from households and live birds markets within the study areas and tested for avian haemosporidian parasites using microscopic analysis of thin blood and buffy coat films, and identification of parasites was based on morphology. The present study found *Plasmodium* (13.8%), *Haemoproteus* (6.8%), and *Leucocytozoon* (0.8%) in a single infection, mixed *Plasmodium* and *Haemoproteus* (2.8%), mixed *Plasmodium* and *Leucocytozoon* (0.3%), and mixed *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (0.3%) in infected chickens. The prevalence of parasites was significantly higher (p -value: 0.0159; $\chi^2 = 5.816$) in males (16.0%) compared to females (8.5%) chickens; significantly higher (p -value: < 0.0001; $\chi^2 = 37.346$) in adults (19.3%) compared with grower (5.3%) chickens; and significantly higher (p -value: < 0.0001; $\chi^2 = 64.347$) in the rainy (21.0%) compared to dry season (3.5%) in the study period. The prevalence of natural infection of avian haemosporidian parasites was higher in chickens sample in households (14.8%) relative to chickens sampled from live birds markets (9.8%); but the correlation is not significant (p -value: 0.1007; $\chi^2 = 2.694$). The present study showed that free-range chickens in Yamaltu Deba, Gombe State, Nigeria have avian haemosporidian parasites, thus providing insight into more conscientious management practices in the country's poultry systems to prevent widespread transmission of the parasites to commercial exotic poultry farms.

Keywords: Haemosporidian; Chickens; *Plasmodium; Haemoproteus; Leucocytozoon*; Yamaltu-Deba

Introduction

Village poultry production systems form an integral part of rural agricultural investments consisting of production of chicken, guinea fowls, pigeons, turkeys, and ducks [1]. In general, taking into consideration the population of birds, biosecurity measures, management and husbandry practices involved, poultry production can be broadly classified as intensive and extensive systems [2]. In an intensive system, birds are usually raised in large numbers in an enclosure, in a controlled environment with sanitation as well as routine treatment when they become ill [3], but in extensive systems, birds are poorly managed, free-range, allowed to scavenge, which can predispose them to disease agents, with hardly any medication when sick [4].

Bacterial, viral, protozoan, and parasitic diseases are seen as one of the most significant threats to poultry production system in African developing countries including Nigeria [5-8]. The adverse effects of parasitic diseases and the economic loss they inflict have been reported on some avian host. It has been reported that avian haemoparasites are pathogenic to domesticated and wild birds

which cause high mortality, reproductive failure, growth retardation, reduced productivity [9]. Avian malaria has been documented to be caused by haemoparasites such as *Plasmodium, Haemoproteus* and *Leucocytozoon* which were reported as the most frequently and worldwide distributed genera of blood parasites [10-11]. In birds, the detection and identification of haemosporidian species may occur via the morphology and morphometry of erythrocyte stages, including the length, width, area, size and number of haemozoin granules [12] and through the amplification and sequencing of DNA [13-15]. Some studies have shown that both methods can have similar sensitivities for detection of these haemoparasites [10,16-17]. Infections of the *Plasmodium* and *Leucocytozoon* species among avian haemoparasites were reported to be responsible for significantly observed acute outbreaks of avian malaria in chickens (*Gallus gallus domesticus*) and other domesticated avian species [18]. *Haemoproteus* species have been reported in domesticated and wild birds, in particular pigeons, and are considered non-pathogenic in infected poultry species, including chickens [19]. The population of infected birds is often seen as asymptomatic carriers and exhibits primarily chronic parasitaemia. Avian haemoparasites are transmitted by specific and non-specific arthropod vectors; unlike in humans, malaria parasites are transmitted by mosquitoes only in the *Anophelinae* subfamily, whereas avian *Plasmodium* species are transmitted by mosquitoes in the *Culicidae* [20]. *Haemoproteus* species is transmitted by biting midges (*Ceratopogonidae*) or louse flies (*Hippoboscidae*) [20], and black flies (Diptera: *Simuliidae*) transmit *Leucocytozoon* species [21]. Recent research has shown that the spread and heterogeneity of avian haemosporidian parasites depends on host-parasite compatibility [22], vector-parasite compatibility [23] and feeding habits for arthropods [24,25]. Available previously published knowledge indicates that the commonly reported avian haemosporidian species have a wide range of avian hosts [26]; thus, accurate techniques and expertise are needed to detect and screen these parasites in a selected host. The aim of this study was therefore to investigate the prevalence of natural infections of avian haemosporidian parasites in free-range chickens in Yamaltu Deba Local Government Area of Gombe State, Nigeria.

Materials and Methods

Study area

This study was carried out in the Yamaltu-Deba Local Government Area, its headquarters is located in the town of Deba (or Deba Habe), southeast of Gombe State, Nigeria. The L.G.A is located approximately 609.5 m above sea level and is located at approximately 10° 12'42''N to 11° 23'11''E with its headquarters, has an area of 1,981 km2, the southern part of Lake Dadin Kowa is located within the region. The climate is characterized by heavy rainfall and high temperatures. The mean temperature varies from 30 - 32^{0}C, the rainfall cycle is unimodal between 700 - 1250 mm and is characterized by distinct dry seasons (October-May) and rainy seasons (June-September) [27].

Study design

A cross-sectional survey design that adopts non-probability sampling techniques was used to conveniently sample five (5) districts, including Dadin Kowa, Deba, Zambuk, Kuri and Kwadon.

Study population

This study included a total of 400 free-range chickens (*Gallus gallus domesticus*) of both sexes (cocks and hens) and diverse age groups. Chickens ages were considered as growers (3 - 4 months) and adults (over 5 months). Chickens are mainly free-range chickens within the study area and were sampled from both households that raise various species of birds and live bird markets with different species of birds. Blood samples were taken from apparently healthy live birds after receiving approval from their owners.

Period of study and blood sample collection

Between September 2016 to November 2017, blood samples were aseptically collected from free-range chickens (*Gallus gallus domesticus*) via the brachial vein (venipuncture). 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. For easy transport to the Department of Veterinary Parasitology and Entomology Research Laboratory, University of Maiduguri, Borno State, Nigeria, slides were carefully packed and arranged in slide boxes.

Microscopic detection of haemoparasites

The slides were stained with Giemsa stain (pH 7.2), raised with distilled water, and allowed to air dry. Stained blood smears were later viewed under a light microscope first at low magnification (100x), and then at high magnification (1000x) oil immersion objective. The haemosporidian parasites schizonts, gametocytes, and trophozoites were examined and classified as described earlier by [28]. The genus stage has been described as avian haemosporidian parasites belonging to the genera *Plasmodium, Haemoproteus,* and *Leucocytozoon*.

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). Differences were considered significant for p-values equal to or less than 0.05.

Results

Table 1 summarizes the results of the prevalence of natural infection of avian haemosporidian parasites in free-range chickens in Yalmaltu Deba Local Government Area of Gombe State, Nigeria. Avian haemosporidian parasites were found in 98 chickens with an overall prevalence rate of 24.5% of the total 400 free-range chickens sampled and examined. The prevalence rate in Dadin Kowa (34.9%) was found to be higher followed by Deba (26.3%), Kuri (21.4%), Kwadon (20.5%) and Zambuk (18.7%) in a descending order of prevalent rate.

Study areas	No. of chickens examined	No. of chickens infected (%)	Prevalence (%)	95% CI LL – UL
Deba	80	21 (26.3)	5.3	17.86 - 36.80
Dadin Kowa	83	29 (34.9)	7.3	25.56 - 45.66
Zambuk	75	14 (18.7)	3.5	11.46 - 28.93
Kuri	84	18 (21.4)	4.5	14.01 - 31.35
Kwadon	78	16 (20.5)	4.0	13.04 - 30.75
Overall	400	98 (24.5)	24.5	20.54 - 28.94

Table 1: Prevalence of Natural infection of Avian Haemosporidian Parasites in free-range Chickens in Yalmaltu Deba Local Government Area, Gombe State, Nigeria.

Key: LL: Lower Limit; UL: Upper Limit; CI: Confidence Interval

The type of natural infection of avian haemosporidian parasites in free-range chickens in Yalmaltu Deba Local Government Area of Gombe State, Nigeria is shown in Table 2. The result shows a higher prevalence of single *Plasmodium* infection (13.8%) compared to *Haemoproteus* infection (6.8%) and *Leucocytozoon* infection (0.8%), while the prevalence of mixed *Plasmodium* and *Haemoproteus* infection (2.8%) was found to be higher compared to mixed *Plasmodium* and *Leucocytozoon* (0.3%) and triple *Plasmodium, Haemoproteus* and *Leucocytozoon* infection (0.3%).

Table 3 summarizes the results of risk factors associated with natural infection of avian haemosporidians in free-range chickens in Yalmaltu Deba Local Government Area, Gombe State, Nigeria. Considering sex as a risk factor, the prevalence of avian haemosporidian in males (16.0%) was found to be relatively higher than in female (8.5%) chickens, and the association between haemosporidian prevalence and chickens age was found to be statistically significant ($p = 0.0159$; $\chi^2 = 5.816$; RR 0.8660). Considering age as a risk factor, however, the prevalence of avian haemosporidian was found to be relatively higher in adults (19.3%) compared to grower chickens (5.3%) and the association between haemosporidian prevalence and chickens age was also found to be statistically significant ($p < 0.0001$; $\chi^2 = 37.346$; RR = 0.6997). The prevalence of avian haemosporidian in the rainy season (21.0%) was found to be higher compared to the dry season (3.5%) of the sampling period; and the association between haemosporidian prevalence and sampling season was also found to be statistically significant ($p < 0.0001$; $\chi^2 = 64.347$; RR = 0.6237). The prevalence of avian haemosporidian was found to be higher in household sampled chickens (14.8%) compared with chickens sampled from live bird markets (9.8%) in the study area; however, the association between haemosporidian prevalence and sampling locations was not statistically significant ($p = 0.1007$; $\chi^2 = 2.694$; RR = 1.105), as summarized in Table 3

Type of Infection	Haemosporidians Encountered	No. of chickens infected N = 400	Prevalence (%)	95% CI LL - UL
Single	*Plasmodium*	55	13.8	10.72 - 17.47
	Haemoproteus	27	6.8	4.68 - 9.4
	Leucocytozoon	3	0.8	0.26 - 2.18
Mixed	*Plasmodium + Haemoproteus*	11	2.8	1.54 - 4.86
	Plasmodium + Leucocytozoon	1	0.3	0.04 - 1.40
	Plasmodium + Haemoproteus + Leucocytozoon	1	0.3	0.04 - 1.40
Overall		98	24.5	

Table 2: Type of Natural infection of Avian Haemosporidian Parasites in free-range Chickens in Yalmaltu Deba Local Government Area, Gombe State, Nigeria.

Key: LL: Lower Limit; UL: Upper Limit; CI: Confidence Interval

Discussion

The findings from this study revealed an overall prevalence of 24.5% of natural infection of avian haemosporidian parasites in free-range chickens in the study area. This result is higher than 11.4% reported in Borno [29], 19.56% reported in Kano [30],

Risk factors	No. of chickens examined	No. of chickens infected (%)	Prevalence (%)	*p*-value	95% CI LL - UL	χ^2	Relative Risk
Sex							
Male	217	64 (29.5)	16.0[a]	0.0159	64.0 - 76.5	5.816	0.8660
Female	183	34 (18.6)	8.5[b]		75.0 - 86.8		
Age (months)							
Adults (> 5)	205	77 (37.6)	19.3[a]	<0.0001	55.5 - 69.1	37.346	0.6997
Grower (3 - 4)	195	21 (10.8)	5.3[b]		84.0 - 93.2		
Season							
Rainy	200	84 (42.0)	21.0[a]	<0.0001	50.9 - 65.0	64.347	0.6237
Dry	200	14 (7.0)	3.5[b]		88.5 - 96.1		
Study Location							
Live Bird Markets	190	39 (20.5)	9.8[a]	0.1007	73.02 - 85.0	2.694	1.105
Households	210	59 (28.1)	14.8[a]		65.3 - 77.8		

Table 3: Risk factors associated with Natural infection of Avian Haemosporidian Parasites in Free-range Chickens in Yalmaltu Deba Local Government Area, Gombe State, Nigeria.

Key: LL: Lower Limit; UL: Upper Limit; CI: Confidence Interval; χ^2 : Chi-square

12.0% reported in Sokoto [31], and 23.2% reported in Benue [2] as opposed to records from similar studies in some parts of the country. The prevalence rate observed in this study, however, is lower than 46.7% reported in Owerri, Imo [32] and 37.7% reported in Nassarawa [4] states. Differences in prevalence rates from various studies could be due to differences in ecological and climatic factors that could affect the breeding of several species of suitable arthropod vectors that can transmit haemoparasites. In addition, higher prevalence rates of avian haemosporidians in scavenging chickens have been recorded in some parts of Africa such as 43.4 % in Ethiopia [33], 79.2 % in Kenya [34], 61.9 % in Uganda [35], 35.0 % in Ghana [36], and 32.0% in Zimbabwe [37]. Such diverse observations showed evidence of the occurrence of avian haemosporidian in scavenging chickens from different parts of the world, all of which were due to the abundance of appropriate vectors that could transmit haemoparasites to chickens.

The prevalence of avian haemosporidian in free-range chickens was found to be relatively higher in Dadin Kowa (7.3%), followed by Deba (5.3%), Kuri (4.5%) and Kwadon (4.0%), whereas Zambuk (3.5%) had the lowest prevalence of avian haemosporidian, based on the study areas sampled during the present study period. The high prevalence rate recorded in Dadin Kowa and Deba may indicate abundance of arthropod vectors in these study areas, but they are the swampiest of the study areas, especially during the rainy season, and Dadin Kowa has the Dadin Kowa dam. These variations in prevalence rates may also be related to differences in vegetation and the availability of stagnant pools of water, swampy or marshy environments and ecological activities that are usually beneficial to the breeding and biology of various vector species such as mosquitoes and many species of flies. The availability and abundance of these arthropod vectors combined with the chickens' scavenging behaviour, may increase their vulnerability. This observation is consistent with Kar *et al*. [38], who documented the role of swampy inhabitants in providing a favorable ecosystem for the breeding of several species of arthropod vectors that play an important role in transmitting haemosporidian and other arthropod-borne diseases among susceptible hosts.

The finding of the present study revealed the presence of three different genera of avian Haemosporidians viz: *Plasmodium*, *Haemoproteu* and *Leucocytozoon* species amongst free-range chickens in the study areas. Mixed infections of two and three combination of these avian haemosporidian parasites were also found in the infected free-range chickens at varying prevalence rates. The findings of this study are consistent with similar research conducted by Hassan *et al*. [4] in Nassarawa and Ogbaje *et al*. [2] in Benue States in Nigeria who have also documented the same three haemoparasites in scavenging chickens. The existence of these three genera of avian haemosporidian parasites in scavenging chickens has been confirmed by several reports from other parts of the world [9,21,39-42].

In the present study, *Plasmodium* species (13.8 %) was the most prevalent followed by *Haemoproteus* (6.8%) and *Leucocytozoon* (0.8%) in a single infection. This finding supported the report by

Igbokwe *et al*. [29], who reported that mosquitoes are endemic in northeastern Nigeria as the major vectors of *Plasmodium* species. In addition, several similar studies have reported *Plasmodium* species in some parts of the world as the most frequently found avian haemosporidian parasite in free-range chickens in their respective study areas [9,33,42]. The findings of the present study are inconsistent with Gimba *et al*. [21], Opara *et al*. [32] and Hassan *et al*. [4], which reported *Haemoproteus* species and Nath and Bhuiyan [25] who reported *Leucocytozoon* species as being the most prevalent in their own studies. It was observed in the present study that free-range chickens are seldom provided with suitable housing or housed in enclosures without arthropod screening nets; this could expose them to bites by several arthropod vectors including mosquitoes and risk of infection. The prevalence rate of *Plasmodium* found during this study is higher than 11.4% previously reported in Maiduguri, Borno State Northeastern Nigeria [29] and 12.0% reported in Sokoto, Sokoto State Northwestern Nigeria [31], but lower than 32.0% in Ibadan, Oyo State Southwestern Nigeria [43], 33.3% in Owerri, Imo State Southeastern Nigeria [32] and 79.2% in Nassarawa State North central Nigeria [4]. The reason for variation in the reported prevalence rates of avian haemosporidian in free-range chickens in these parts of the country might partly be attributed to the variation in ecologic and climatic factors and abundance of vectors.

The findings of the present study revealed the prevalence of single *Haemoproteus* infection as 6.8%, which is relatively higher than 0.9%, 0.8%, 2.5% and 3.3% from Ethiopia, Malaysia, Bangladesh, and Uganda, as reported by Sabuni *et al*. [34], Gimba *et al*. [21], Nath and Bhuiyan [25], and Nakayima *et al*. [42] respectively. The present study also revealed 0.8% prevalence of single *Leucocytozoon* infection in free-range chickens, which was found to be below 2.3% in Uganda reported by Nakayima *et al*. [42].

Mixed infection with two avian haemosporidian parasites which comprises of *Plasmodium* and *Haemoproteus* was found at a prevalence rate of (2.8%). This finding agrees with Nath and Bhuiyan [25] and Naqvi *et al*. [44] who in their respective studies have reported mixed *Plasmodium* and *Haemoproteus* species infection in free-range chickens. However, our result is lower than 3.5% reported by Sabuni *et al*. [34] respectively, but higher than 0.5% reported by Nath and Bhuiyan [25]. The findings of the present study also revealed mixed *Plasmodium* and *Leucocytozoon* species infection with a prevalence rate of 0.3%, which was lower than 9.33%, 34.2% and 6.5% reported previously by Sadiq *et al*. [43], Sabuni *et al*. [34] and Nath and Bhuiyan [25] respectively. The present study also found mixed infection involving three species of avian haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) in infected chickens at a prevalence rate of 0.3%, which is lower than 4.0% reported by Sadiq *et al*. [43]. The findings of the present study are also consistent with reports by Hellgren *et al.* [12] in Sweden, Valkiûnas *et al.* [35] in Uganda, Sabuni *et al.* [34] in Kenya, Gimba *et al.* [21] in Malaysia, Nath and Bhuiyan [25] in Bangladesh, Naqvi *et al.* [44] in Pakistan, Hassan *et al.* [4] in Nigeria who have documented in their various studies various combinations of these three genera of avian haemosporidian parasites in the free-range chickens. The difference in the reported prevalence rates of mixed haemosporidian infections in scavenging chickens may partly be due to variation in geographic distribution of arthropod vectors.

The results of this study showed a higher prevalence in males (16.0%) compared to female chickens (8.5%) that was statistically significant ($p < 0.0001$) and can be attributed to the facts that cocks anatomically have larger combs and wattles that are richly vascularized with blood vessels, which can attract blood sucking arthropods that can transmit haemosporidian parasites to blood vessels during blood meal. This finding is consistent with Opara *et al*. [32], Lawal *et al*. [45], Hassan *et al*. [4] and Ogbaje *et al*. [2], who also reported a higher prevalence of haemosporidian parasites in males compared to female free-range chickens in Owerri, Maiduguri, Nassarawa and Markudi, respectively, in Nigeria. The finding of this study also confirmed previous reports by Sabuni *et al*. [34] in Kenya, Hasan *et al*. [9] in Bangladesh, Etisa *et al*. [33] in Ethiopia, which also reported a high prevalence of haemosporidian parasites in free-range chicken cocks in their respective studies compared to the hens. In contrast, Naqvi *et al*. [44] in Pakistan and Nath and Bhuiyan [25] in Bangladesh indicated that the hens have higher prevalence rates of infection with avian haemosporidian parasites compared to the cocks in scavenging chickens. There are generally conflicting reports about the frequency and effect of host sex on the prevalence rate of various avian haemosporidian parasites in several bird species [15].

In the present study, the prevalence of avian haemosporidian parasite infection based on the age of free-range chickens showed a higher prevalence in adults (19.3%) compared to growers (5.3%), which was statistically significant ($p<0.0001$). This could be attributed to the behavior of adult free-range chickens who roam far away in search of mating mates and also scavenge for food in or near unhygienic environments, which may predispose them to high risks of contact with vectors of arthropods, while young ones are usually found near their roosting areas, away from vectors or predators and under the care of parent birds. Adult chickens also appear to have more prominent and well-developed combs and

wattles that are well vascularized and provide abundant predilection sites for arthropod vectors, consistent with Abdul Momin *et al*. [39] in Bangladesh and Etisa *et al*. [33] in Ethiopia, which also reported high prevalence ($p<0.05$) of avian haemosporid parasite infection in adult chickens compared to growers. Higher prevalence of avian haemoparasites may also be attributed to longer periods of exposure to arthropod vectors in adult chickens [46]. However, this finding contradicts that of Sabuni *et al*. [34] in Kenya and Naqvi *et al.* [44] in Pakistan who reported a relatively high prevalence of infection with avian haemosporidian parasites in growers compared to adult chickens, but the difference was not statistically significant ($p > 0.05$).

The results of this season-based study showed a significantly higher prevalence ($p < 0.0001$) of avian haemosporidian parasites during the rainy season (21.0%) than in the dry season (3.5%). The finding could be attributed to the fact that the rainy season is usually the most favorable season, providing suitable conditions, such as temperature and humidity, for the breeding of most arthropod vectors, such as mosquitoes and other biting flies. This can be supported by the high prevalence in human and animal populations of parasitic infections such as malaria and trypanosomosis during the rainy season in Nigeria [47]. The high prevalence recorded in this study is consistent with Igbokwe *et al*. [29], who also reported a high prevalence of avian haemosporidian parasites during the rainy season in Maiduguri, Nigeria, but contrasted with Nath and Bhuiyan [25] reports in hilly areas of Bangladesh, which reported a prevalence of 60.6% in summer, 36.7% in rainy and 23% in winter seasons. In addition to rainfall and variations in habitat composition, prevalence rates may be affected by the proximity of vector breeding sites, relative levels of host resistance, local temperature differences, sample collection time, sample collection effort and host age among others [19]. Vector breeding rates are increasingly high in the Northeastern, Nigerian rainy seasons [29], and this important role of seasonal impact on vectors and haemoparasites could be used as a vital tool in the institution of preventive and control measures [19].

This present study showed a higher prevalence of avian haemosporidian parasites in chickens sampled from household (14.8%) than those sampled from the live birds markets (9.8%), but there was no statistically significant difference in the prevalence rate of infections between the sampling locations. That means the sampling locations is not a risk factor for the chickens to become infected with haemosporidian parasites. This finding is not surprising, as most household chickens often scavenge, making them more vulnerable to vectors than those in the market are restricted and usually sold for meat or restocking. This finding is in line with previous reports by Lawal *et al*. [45], Opara *et al*. [32] and Hassan *et al*. [4], who also reported that the sample location is not a risk factor for haemosporidian infection; however, susceptible chicken may be infected, irrespective of the sampling site, as long as there is availability of infected arthropod vectors.

Conclusion and Recommendation

In Yamaltu Deba LGA of Gombe State, Nigeria, three avian haemosporidian parasites (*Plasmodium, Haemoproteus* and *Leucocytozoon* species) were found to infect naturally free-range chickens with an overall prevalent rate of 24.5%. Infected chickens where found to harbor either a single or mixed infection of these haemosporidian parasites. The most prevalent haemosporidian parasite in infected chickens was found to be *plasmodium*. Variations in the prevalence of avian haemosporidian parasites were observed in relation to their age, bird sex and sampling season, with the prevalence 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. Sample location of chickens in the present study area was not a risk factor for the determination of natural infection of avian haemosporidian parasite infection in free-range chickens. Therefore, further study to characterize the avian haemosporidian in chickens and other poultry species to their species level is recommended using molecular detection tools to further understand the epidemiology of the disease in the study area.

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