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Effects of avian malaria parasites infections on hematological and biochemical parameters in village chickens in Gombe state, Nigeria

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ABSTRACT

Aim: The study was aimed to examine avian haemoparasites and their effects on haematological parameters in village chickens in Gombe State, Nigeria.

Method and materials: Blood samples from village chickens across 8 Local Government Areas were collected. All samples were transported under controlled conditions to maintain sample integrity. Hematological and biochemical analyses were performed following standardized protocols.

Results: The overall prevalence of avian haemoparasites was 19.6% (356/1820), with *Plasmodium* at 13.9% (253/1820), *Haemoproteus* at 2.6% (47/1820), *Leucocytozoon* at 0.4% (8/1820), and mixed infections of *Plasmodium* and *Haemoproteus* at 2.6% (48/1820). Infected chickens exhibited significant changes in haematological parameters. The mean±SD PCV (%) was lower in chickens infected with *Plasmodium* (25.6±6.4), *Haemoproteus* (27.1±5.2), *Leucocytozoon* (25.0±3.0), and mixed infections (24.5±5.2) compared to uninfected chickens (32.0±6.8). Similarly, Hb concentration (g/dl) was lower in infected chickens, with values for *Plasmodium* (10.0±2.8), *Haemoproteus* (10.3±2.4), *Leucocytozoon* (11.0±1.8), and mixed infections (9.8±2.6) versus uninfected chickens (11.4±2.3). RBC counts ($x10^6$/µl) also decreased in infected chickens, indicating normocytic normochromic anaemia. Heterophils and lymphocyte values increased in infected chickens. Biochemical analysis showed significantly ($p < 0.05$) lower levels of blood glucose, total plasma protein, serum albumin, serum globulin, and alanine aminotransferase in infected chickens, especially those with *Plasmodium* or mixed infections. *Haemoproteus*-infected chickens had higher total plasma protein and serum albumin levels than uninfected chickens, while *Leucocytozoon*-infected chickens showed generally lower, but not statistically ($p > 0.05$) significant, values.

Conclusion: In conclusion, avian haemoparasites are prevalent among village chickens in Gombe State, significantly affecting their haematological and biochemical profiles, leading to anaemia and altered biochemical parameters. Village chicken farmers should be informed about the impact of avian malaria on poultry productivity, and further research should be conducted to evaluate the prevalence and epidemiology of avian haemoparasites in other poultry species and regions of Nigeria.

Keywords: Avian malaria, Anaemia, *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, Avian Biochemistry and Haematology.

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Introduction

Despite great potentials and opportunities, village poultry production system is perpetually threatened by diseases causing heavy losses (Ekiri *et al*., 2021; Otte *et al*., 2021). Infectious diseases significantly limit village chicken productivity in many developing countries, including Nigeria (Grace *et al*., 2024). Surprisingly, parasitic diseases receive less attention despite their role in reducing productivity and increasing mortality (Grace *et al*., 2024).

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Unlike infectious diseases such as Newcastle disease, which result in severe outbreaks and high rates of morbidity and mortality, parasitic diseases often go unnoticed (Grace *et al*., 2024).

Haemosporidian parasites, emerging pathogens increasingly reported in poultry species worldwide in recent times, occur irrespective of climatic barriers (Valkiûnas, 2005; Ghaemitalab *et al*., 2021). The most common haemoparasites found in avian species, including *Plasmodium, Haemoproteus, Leucocytozoon*, and *Trypanosoma*, are prevalent in warm continents and particularly common in undisturbed tropical regions of developing countries (Villalva-Pasillas *et al*., 2020;

Muriel *et al*., 2021; Martín-Maldonado *et al*., 2023) and are spread by blood-sucking Dipteran (Valkiûnas, 2005; Valkiūnas and Iezhova, 2022; Tembe*et al*., 2023). While avian haemosporidian infections typically do not lead to significant mortality, they can affect host fitness, stress levels, disease susceptibility, survival rates, immunological response, and reproductive success, potentially contributing to population decline and extinction (Rodriguez *et al*. 2021; Cruz *et al*., 2024).

Haematological studies play a crucial role in diagnosing and monitoring various diseases, as well as assessing blood damage (Dadkhah*et al*., 2022). Research indicates that infections like *Plasmodium* and *Haemoproteus* can significantly impact the survival and health of hosts, leading to alterations in haematological parameters (Gutiérrez-Ramos and Acevedo 2024). Blood serves as an indicator of health status of animals exposed to infectious agents (Linhart *et al*., 2022). Notably, studies have shown that certain haematologic parametersare altered in birds infected with parasites (Malvat *et al*., 2020). The current investigation aims to examine avian haemoparasites and their effects on haematological parameters in village chickens in Gombe State, Nigeria.

Materials and Methods

The study was carried out in Gombe State, Northeastern Nigeria. Gombe State is located between latitude 9^0 30′ and 12^0 3′ N and longitude 8^0 45′ and 11^0 45′ E (Anon., 2009), and has a mean annual rainfall of 818.5mm, a mean maximum temperature of 37^0C, and a mean minimum temperature of 12^0C. The poultry population in Gombe State is approximately put as 508,305 comprising of 462,000 backyard poultry and 46,305 exotic poultry (Adene and Oguntade, 2006). Rural and urban areas within eight (8) out of the eleven (11) Local Government Areas of the State were visited for sample collection. The 8 LGAs sampled were: - Gombe, Akko, Funakaye, Kwami, Dukku, Yamaltu-Deba, Kaltungo and Balanga. Three (3) villages/districts were visited from each LGA, and three (3) village chicken farmers' households were visited from each village/district. During the period of the study, a total of 1820 chickens were sampled. The inclusion criteria are that any farmer's household with at least five (5) chickens and have consented to participate in the study were sampled. Chickens of different ages and sexes were sampled from each household.

Study Design: A cross-sectional study was designed to determine the prevalence and effects of haemosporidian parasites infection on the haematological and biochemical parameters of village in Gombe State, Nigeria. The study covered broadly two sampling periods. The first period targeted rainy season while the second period targeted dry season. This was to enable us to obtain a fair representation of situation within the study population. Samples were drawn from the study population using convenient sampling technique. The study was carried out betweenNovember, 2016 to September, 2017following anecdotal reports of decreased productivity and increased parasite burden among village chickens in the study area.

Blood Sample Collection Preservation: A total of 3 ml of blood was aseptically collected from each bird via venipuncture of the brachial vein using sterile 5ml syringes and 21-gauge needles. Blood samples were immediately transferred into two different blood sample bottles first to an ethylenediaminetetraacetic acid (EDTA) tubes to prevent coagulation and ensure accurate hematological analysis and the other a plain blood sample bottle without anticoagulant to ensure coagulation and serum harvest. The collected blood samples in anticoagulant bottles were transported to the Hematology Laboratory of the Department of Veterinary Pathology, University of Maiduguri for haematological parameters analysis while the blood samples collected in plain bottles were transported to Hematology Laboratory, University of Maiduguri Teaching Hospital for biochemistry parameter analysis. All samples were transported under controlled conditions to maintain sample integrity.

Preparation of Blood Smears for Identification of Haemoparasites: Thin blood film and buffy coat smears were made from each blood sample in duplicates on two different clean dry slides as described by Mello *et al*. (2014) and World Health Organization (WHO) (2016) and were left for few minutes to air dry and then labeled appropriately. The slides were then fixed with methanol for five (5) minutes, allowed to air dry again, packaged and then later staining with diluted 10% Giemsa stain 7.4 according to the standard procedures described by Thrall (2004), Ribeiro *et al.* (2005) and Zajac and Conboy (2012). The slides were later viewed at low magnification (×40) and at high magnification

(×100) using the light microscope under oil immersion for the presence of intracellular and extracellular blood parasites and their gametocytes as previously described by Valkiûnas (2005), Valkiûnas *et al*. (2008b) and Akinpelu (2008). Identification of parasites was based on their morphology and compared to reference plates (Taylor *et al*., 2007).

Determination of Haematological Parameters: Hematological parameters, including packed cell volume (PCV), total and differential white blood cell counts (WBC), hemoglobin (Hb) concentration, and red blood cell counts (RBC), as well as biochemical analysis which include blood glucose, total plasma protein, total serum albumin, total serum protein, total serum globulin, and serum alanine aminotransferase (ALT) were all determined using standard procedures. All hematological and biochemical analyses were performed following standardized protocols described by Nabi *et al*. (2022), Joshi *et al*. (2023) and Lebednikaitė *et al.* (2024). Quality control measures were implemented to ensure the accuracy and precision of the results. The obtained hematological and biochemistry values were compared to the normal range of reference values for hematological/biochemistry parameters and indices specific to village chickens, as described by Bounous and Stedman (2000.

Statistical analysis: Statistical analyses of the haematological data were performed using SPSS (version 21.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics, including mean, standard deviation, and range, were computed for each hematological parameter. Analysis between the means of haematological parameter of birds infected and the means for the birds that were not infected was carried out using t-test while difference between the groups means was analyzed using ANOVA with with p-value $p < 0.05$ considered statistically significant. Before conducting each Student t-test, a Folded F-test was employed to assess the equality of variances between the two groups. Subsequently, the choice of t-test method (Pooled or Satterthwaite) was determined based on the results of the F-test. The pooled method was applied when equal variances were assumed, whereas the Satterthwaite method was adopted in cases of unequal variances (p-value of the Folded F-test <0.05). Additionally, a multiple comparison test (such as Turkey's test) was conducted as a post hoc analysis.

Results and Discussion

Prevalence of Avian Malaria Parasites Infection in Village Chickens Based on Parasite Genera

The results of microscopy examinations of blood smears for the presence of avian haemoparasites in village chickens in Gombe State was shown (Table1). The results revealed that *Plasmodium* (13.90) was the most prevalent haemoparasites followed by mixed infection of *Plasmodium*+*Haemoproteus* (2.64%) and *Haemoproteus* (2.58%) while *Leucocytozoon* (0.44%) was the least prevalent haemoparasites detected.

Haematological Indices and Serum Biochemistry in Village Chickens Infected with Avian Malaria Parasites

The results of changes in haematological parameters and indices associated with haemoparasitosis in village chickens were shown (Table 2). The hematological parameters of village chickens infected with different avian hemoparasites, including *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and co-infection with *Plasmodium* and *Haemoproteus*, were compared with uninfected chickens (normal values).

Uninfected chickens had a mean PCV of 32.0 ± 6.8% (22 – 35%). However, chickens infected with *Plasmodium* had a significantly lower PCV (25.6 ± 6.4%), followed by *Haemoproteus* (27.1 ± 5.2%), *Leucocytozoon* (25.0 ± 3.0%), and co-infection of *Plasmodium* and *Haemoproteus* (24.5 ± 5.2%). Hemoglobin levels were significantly reduced in infected chickens, with uninfected chickens having a mean of 11.4 ± 2.3 g/dL (7 – 13 g/dL). Chickens infected with *Plasmodium* had a mean Hb of 10.0 ± 2.8 g/dL, *Haemoproteus* 10.3 ± 2.4 g/dL, *Leucocytozoon* 11.0 ± 1.8 g/dL, and co-infection resulted in the lowest mean Hb of 9.8 ± 2.6 g/dL. A significant reduction in RBC ($x10^6$ /µl) count was observed in infected chickens. The uninfected group had a mean RBC count of 2.9 ± 0.9 $x10^6$ /µl (2.5 – 3.5 $x10^6$ /µl), while the lowest count was recorded in co-infected chickens (2.3 ± 0.5 $x10^6$ /µl), followed by *Plasmodium* (2.4 ± 0.6 $x10^6$ /µl), *Haemoproteus* (2.6 ± 0.6 $x10^6$ /µl), and *Leucocytozoon* (2.4 ± 0.2 $x10^6$ /µl). No significant differences were observed in MCV values among infected and uninfected chickens. The MCV for all groups remained consistent at approximately 110 ± 15 fL (90 – 140 fL). Infected chickens displayed significantly higher MCH levels compared to uninfected ones, with a mean of 43 ± 8.1 Pg in *Plasmodium* infections, 41 ± 7.3 Pg in *Haemoproteus*, 46 ± 6.4 Pg in *Leucocytozoon*, and 43 ± 6.4 Pg in co-

infections, compared to 39 ± 5.8 Pg in uninfected chickens. Similarly, MCHC values were significantly higher in infected chickens, particularly in those with *Leucocytozoon* (44 ± 4.3 g/dL) and co-infections (40 ± 6.0 g/dL), compared to the uninfected group (36 ± 4.7 g/dL).

Infected chickens had elevated WBC ($x10^3$ /µl) counts compared to uninfected chickens, which had a mean of 16.3 ± 2.8 $x10^3$ /µl (12 – 30 $x10^3$ /µl). The highest WBC count was observed in chickens infected with *Leucocytozoon* (23.3 ± 2.9 $x10^3$ /µl), followed by *Plasmodium* (21.1 ± 1.7 $x10^3$ /µl), *Haemoproteus* (20.5 ± 1.5 $x10^3$ /µl), and co-infected chickens (21.5 ± 1.8 $x10^3$ /µl).

No significant differences were observed in heterophil ($x10^3$ /µl) counts among infected chickens. Uninfected chickens had a mean heterophil count of 4.7 ± 1.2 $x10^3$ /µl, while infected groups had slightly elevated values. Lymphocyte ($x10^3$ /µl) counts were significantly higher in infected chickens, with the highest count recorded in *Leucocytozoon*-infected chickens (12.5 ± 1.8 $x10^3$ /µl), compared to the uninfected group (9.9 ± 2.0 $x10^3$ /µl). Monocyte ($x10^3$ /µl) counts were significantly elevated in infected chickens, with the highest count seen in co-infected chickens (2.4 ± 0.6 $x10^3$ /µl), compared to 1.0 ± 0.6 $x10^3$ /µl in uninfected chickens. Eosinophil ($x10^3$ /µl) counts were significantly increased in infected chickens, particularly in those with *Leucocytozoon* (2.8 ± 0.5 $x10^3$ /µl) and co-infections (2.2 ± 0.6 $x10^3$ /µl), compared to uninfected chickens (0.7 ± 0.6 $x10^3$/µl).

The comparative haemogram of village chickens from Gombe State, Nigeria was showed notable differences in haematological parameters (Table 3) between uninfected chickens and those infected with haemoparasites (*Plasmodium, Haemoproteus, Leucocytozoon,* and mixed infections of *Plasmodium* + *Haemoproteus*). Uninfected chickens had a mean ± SD of 31.7% ± 7.3, while infected chickens showed significant reductions, particularly in chickens with *Haemoproteus* and *Leucocytozoon* infections, where PCV values dropped to 22.5% ± 3.0 and 22.0% ± 2.0, respectively. Uninfected chickens had a mean ± SD of 11.4 g/dl ± 2.6. Chickens infected with *Plasmodium* + *Haemoproteus* had the most pronounced decrease, with values ranging from 8.4 g/dl ± 0.9 to 8.9 g/dl ± 0.5, indicating significant anaemia. The RBC count for uninfected chickens was 2.9 $x10^6$ /µl ± 1.1. Infected chickens, especially those with *Haemoproteus* and *Leucocytozoon*, showed decreased RBC counts, with the lowest recorded in *Plasmodium* + *Haemoproteus* infected chickens (2.1 $x10^6$ /µl ± 0.3).

Uninfected chickens had a Mean Corpuscular Volume (MCV) of 110 fL ± 16. There were no significant differences in MCV across infected chickens, with most showing values close to the uninfected range. The Mean Corpuscular Haemoglobin (MCH) was elevated in chickens with haemoparasite infections, particularly in *Plasmodium* (44 pg ± 8.1) and *Leucocytozoon* (45 pg ± 7.3), compared to uninfected chickens (40 pg ± 6.3). Mean Corpuscular Haemoglobin Concentration (MCHC) was significantly higher in infected chickens, especially in mixed infections of *Plasmodium* + *Haemoproteus* (42 g/dL ± 6.4), compared to the uninfected group (36 g/dL ± 5.1).

Infected chickens showed elevated White Blood Cells (WBCs) counts. Chickens with *Leucocytozoon* infections had counts as high as 22.3 $x10^3$ /µl ± 2.1, compared to uninfected chickens with 16.4 $x10^3$ /µl ± 3.0. The mean heterophil count was relatively consistent between infected and uninfected chickens, with minor fluctuations across the groups. Infected chickens, particularly those with *Leucocytozoon* and mixed infections, had elevated lymphocyte counts compared to uninfected chickens (9.9 $x10^3$ /µl ± 2.1). The highest lymphocyte counts were observed in chickens with *Leucocytozoon* (12.6 $x10^3$ /µl ± 1.7). Significant increases in monocyte counts were observed in all infected groups, especially in chickens with *Leucocytozoon* infections (2.7 $x10^3$ /µl ± 0.2) compared to uninfected chickens (1.1 $x10^3$ /µl ± 0.7). Infected chickens showed significantly elevated eosinophil counts, with chickens infected with *Leucocytozoon* showing the highest values (2.9 $x10^3$ /µl ± 0.5), compared to uninfected chickens (0.8 $x10^3$ /µl ± 0.6).

Summarizes key was recorded of serum biochemistry parameters (Table 4) in village chickens, comparing uninfected chickens with those infected by various avian haemoparasites, including *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and dual infections of *Plasmodium* and *Haemoproteus*.Blood glucose levels are significantly lower in chickens infected with *Plasmodium* (191.9 mg/dl) and *Plasmodium* + *Haemoproteus* (188.6 mg/dl) compared to the uninfected group (195.3 mg/dl). However, the levels in chickens infected with *Haemoproteus* (192.3 mg/dl) and *Leucocytozoon*

(193.6 mg/dl) are comparable to those of uninfected chickens. Infection with *Plasmodium* and *Plasmodium* + *Haemoproteus* results in significantly lower total plasma protein levels (2.663 g/dl and 2.525 g/dl, respectively) compared to uninfected chickens (2.859 g/dl). On the other hand, *Haemoproteus* and *Leucocytozoon* infections have a lesser effect on this parameter, showing levels close to uninfected chickens. *Plasmodium* and *Plasmodium* + *Haemoproteus* infections significantly lower total serum protein levels (2.803 g/dl and 2.706 g/dl, respectively). Conversely, *Haemoproteus* and *Leucocytozoon* infections do not significantly impact this parameter, with values nearly identical to those of uninfected chickens. Serum albumin levels decrease significantly in *Plasmodium*-infected and *Plasmodium* + *Haemoproteus*-infected chickens (2.821 g/dl and 2.598 g/dl, respectively). Chickens infected with *Haemoproteus* or *Leucocytozoon* maintain levels close to the uninfected group. *Plasmodium* and *Plasmodium* + *Haemoproteus* infections lead to a significant reduction in serum globulin (0.971 g/dl and 0.835 g/dl, respectively). The effects of *Haemoproteus* and *Leucocytozoon* infections are milder, with values still lower than uninfected chickens. Serum ALT is significantly lower in *Plasmodium*-infected and *Plasmodium* + *Haemoproteus* - infected chickens compared to uninfected chickens. However, the levels in chickens infected with *Haemoproteus* and *Leucocytozoon* are not significantly affected, remaining within the normal range.

Table 1. Prevalence of avian malaria parasite infections in village chickens by Genera in Gombe State, Nigeria

Description	Information	Number (%) infected with:			
		Plasmodium	*Haemoproteus*	*Leucocytozoon*	*Plasmodium* + *Haemoproteus*
Over all		253 (13.90)	47 (2.58)	8 (0.44)	48 (2.64)
Study location - wise	Markets	71 (3.90)[a]	25 (1.37)[a]	3 (0.16)[a]	21 (1.15)[a]
	Households	182 (10.0)[b]	22 (1.21)[b]	5 (0.27)[a]	27 (1.48)[a]

Table 2. Hematological parameters and indices associated with avian malaria parasites in village chickens in Gombe State, Nigeria

Haematological Parameters/Indices	Mean ± SD (range) for Uninfected Village Chickens (Normal Values)	Mean±SD values for Village Chickens infected with;			
		Plasmodium n = (253)	*Haemoproteus* n = (47)	*Leucocytozoon* n = (8)	*Plasmodium* + *Haemoproteus* n = (48)
PCV (%)	32.0[a]±6.8 (22 – 35)	25.6[b]±6.4	27.1[b]±5.2	25.0[b]±3.0	24.5[b]±5.2
Hb (g/dl)	11.4[a]±2.3 (7 – 13)	10.0[b]±2.8	10.3[b]±2.4	11.0[a,b]±1.8	9.8[b]±2.6
RBC ($x10^6/\mu l$)	2.9[a]±0.9 (2.5 – 3.5)	2.4[b]±0.6	2.6[b]±0.6	2.4[a,b]±0.2	2.3[b]±0.5
MCV (fL)	110[a]±15 (90 – 140)	110[a]±20	110[a]±18	110[a]±14	110[a]±15
MCH (Pg)	39[a]±5.8 (33 – 47)	43[b]±8.1	41[a,b]±7.3	46[b]±6.4	43[b]±6.4
MCHC (g/dL)	36[a]±4.7 (26 – 35)	39[b]±6.1	38[a]±4.5	44[b]±4.3	40[b]±6.0
WBC ($x10^3/\mu l$)	16.3[a]±2.8 (12 – 30)	21.1[b]±1.7	20.5[b]±1.5	23.3[b]±2.9	21.5[b]±1.8
Heterophils ($x10^3/\mu l$)	4.7[a]±1.2 (3 – 6)	5.1[b]±1.3	4.8[a,b] ±0.8	5.3[a,b] ±0.8	4.9[a,b]±0.9
Lymphocyte ($x10^3/\mu l$)	9.9[a]±2.0 (7 – 17.5)	11.9[b]±2.1	11.4[b]±1.7	12.5[b]±1.8	11.9[b]±1.6
Monocyte ($x10^3/\mu l$)	1.0[a]±0.6 (0.1 – 2)	2.2[b]±0.6	2.1[b]±0.5	2.6[b]±0.4	2.4[b]±0.6
Eosinophil($x10^3/\mu l$)	0.7[a]±0.6 (0 – 1)	2.0[b]±0.7	2.1[b]±0.5	2.8[b,c]±0.5	2.2[b,c]±0.6

[a,b,c] Values with different superscripts are significantly ($p < 0.05$) different

Table 3. Comparative hemogram of village chickens in Gombe State, Nigeria, infected with avian malaria parasites

Haematological Parameters//Indices	Mean ± SD (range) for Uninfected Village Chickens n = (1464) (Normal Range)		Mean±SD values for Village Chickens Infected with;							
			Plasmodium n = (253)		*Haemoproteus* n = (47)		*Leucocytozoon* n = (8)		*Plasmodium + Haemoproteus* n = (48)	
	A n = (956)	B n = (508)	A n = (182)	B n = (71)	A n = (22)	B n = (25)	A n = (5)	B n = (3)	A n = (27)	B n = (21)
PCV (%)	31.7^{a}±7.3 (22 – 35)	32.5^{b}±5.6 (22 – 35)	26.9^{a}±6.9	22.5^{b}±3.0	30.1^{a}±5.4	24.4^{b}±3.2	26.8^{a}±1.6	22.0^{b}±2.0	25.5^{a}±6.3	23.3^{a}±2.9
Hb (g/dl)	11.4^{a}±2.6 (7 – 13)	$11.^{a}$±1.8 (7 – 13)	10.8^{a}±2.9	8.1^{b}±1.1	11.9^{a}±2.2	8.9^{b}±1.5	12.3^{a}±0.3	8.9^{b}±0.5	10.9^{a}±2.9	8.4^{b}±0.9
RBC ($x10^{6}$/µl)	2.9^{a}±1.1 (2.5 – 3.5)	3.0^{b}±0.5 (2.5 – 3.5)	2.4^{a}±0.6	2.1^{b}±0.4	2.8^{a}±0.6	2.4^{b}±0.5	2.4^{a}±0.2	2.3^{a}±0.3	2.3^{a}±0.5	2.1^{b}±0.3
MCV (fL)	110^{a}±16 (90 – 140)	110^{a}±11 (90 – 140)	110^{a}±20	110^{a}±20	110^{a}±22	100^{a}±13	110^{a}±.4.2	98^{a}±23	110^{a}±16	110^{a}±14
MCH (Pg)	40^{a}±6.3 (33 – 47)	38^{a}±4.3 (33 – 47)	44^{b}±8.1	39^{a}±7.0	44^{b}±8.6	38^{a}±3.9	50^{b}±2.3	39^{a}±3.6	45^{b}±7.3	40^{a}±3.5
MCHC (g/dL)	36^{a}±5.1 (26 – 35)	35^{a}±3.7 (26 – 35)	$40^{b,c}$±6.7	$36^{a,b}$±2.7	40^{b}±5.4	36^{a}±2.9	46^{b}±1.9	$41^{b,c}$±6.0	42^{b}±6.4	$36^{a,b}$±2.8
WBC ($x10^{3}$/µl)	16.4^{a}±3.0 (12 – 30)	16.0^{b}±2.5 (12 – 30)	21.4^{a}±1.9	20.4^{b}±0.9	21.1^{a}±1.5	20.0^{b}±1.3	25.0^{a}±2.0	20.4^{b}±0.9	22.3^{a}±2.1	20.6^{b}±0.7
Heterophils ($x10^{3}$/µl)	4.6^{a}±1.2 (3 – 6)	4.8^{b}±1.1 (3 – 6)	5.1^{a}±1.4	5.0^{a}±1.1	4.6^{a}±0.8	5.0^{a}±0.7	5.6^{a}±0.9	4.9^{a}±0.6	5.0^{a}±0.9	4.8^{a}±0.8
Lymphocyte ($x10^{3}$/µl)	9.9^{a}±2.1 (7 – 17.5)	9.9^{a}±1.6 (7 – 17.5)	12.1^{a}±2.1	11.3^{b}±1.9	12.1^{a}±1.7	10.7^{b}±1.3	13.8^{a}±0.6	10.5^{b}±0.6	12.6^{a}±1.7	11.1^{b}±1.0
Monocyte ($x10^{3}$/µl)	1.1^{a}±0.7 (0.1 – 2)	0.8^{b}±0.3 (0.1 – 2)	2.2^{a}±0.6	2.2^{a}±0.6	2.1^{a}±0.5	2.1^{a}±0.5	2.7^{a}±0.2	2.5^{a}±0.5	2.4^{a}±0.6	2.3^{a}±0.6
Eosinophil ($x10^{3}$/µl)	0.8^{a}±0.6 (0 – 1)	0.5^{b}±0.3 (0 – 1)	2.0^{a}±0.6	2.0^{a}±0.7	2.2^{a}±0.6	2.1^{a}±0.5	2.9^{a}±0.5	2.5^{a}±0.5	2.3^{a}±0.6	2.2^{a}±0.7

NB: Values with different superscripts ^{a,b} are significantly ($p < 0.05$) different; Keys: A = Household; B = Market; n = Number of birds examined

Table 4: Serum biochemistry parameters associated with avian malaria parasite infections in village chickens in Gombe State, Nigeria

Biochemical parameters	Mean ± SD (range) for Uninfected Village Chickens (Normal Values)	Mean±SD values for Village Chickens infected with;			
		Plasmodium n = (253)	*Haemoproteus* n = (47)	*Leucocytozoon* n = (8)	*Plasmodium* + *Haemoproteus* n = (48)
Blood glucose (mg/dl)	195.3^{a} ± 3.489 (197 – 299)	191.9^{b} ± 0.6010	192.3^{b} ±0.4150	193.6^{a} ±1.449	188.6^{b} ± 2.544
Total plasma protein (g/dl)	2.859^{a} ± 0.0523 (3.0 – 4.0)	2.663^{b} ±0.146	2.896^{b} ±0.0770	2.894^{a} ±0.088	2.525^{b} ±0.246
Total serum protein (g/dl)	3.857^{a} ±0.192 (3.0 – 6.0)	2.803^{b} ±0.0997	3.856^{a} ±0.0329	3.829^{a} ±0.0164	2.706^{b} ±0.149
Total serum albumin (g/dl)	3.133^{a} ±0.054 (3.28–3.80)	2.821^{b} ±0.045	3.182^{b} ±0.061	3.181^{a} ±0.077	2.598^{b} ±0.289
Total serum globulin (g/dl)	1.281^{a} ±0.040 (1.15 – 1.53)	0.971^{b} ±0.023	1.184^{b} ±0.035	1.133^{b} ±0.093	0.835^{b} ±0.015
Serum ALT (IU/L)	10.88^{a} ± 0.098 (10.6 – 11.9)	9.781^{b} ±0.106	10.65^{a} ±0.152	10.84^{a} ±0.047	9.735^{b} ±0.086

[a,b] Values with different superscripts are significantly ($p < 0.05$) different; ALT = alanine amino transferase

The study identified *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* as prevalent haemoparasites in village chickens, indicating the presence of suitable vectors in the region. Boonchuay *et al*. (2023) highlighted that these parasites thrive in environments with abundant arthropod vectors. Mixed infections with *Plasmodium* and *Haemoproteus* were common, consistent with earlier reports (Wamboi *et al*., 2020; Duc *et al*., 2021; Tembe *et al*., 2023; Talbott and Ketterson, 2023). Variations in haemoparasite species across studies may result from differences in vector abundance, detection methods, husbandry practices, and ecological factors.

Infected chickens showed decreased PCV, Hb, and RBC, indicating anemia, which aligns with findings by Wamboi *et al*. (2020) and Jubril *et al*. (2021). Haemoparasites have been known to cause anemia by destroying RBCs (Townsend *et al*., 2018). Despite this, MCV values remained normal across infected and uninfected chickens, suggesting a normocytic blood picture. This finding concurs with Umar *et al*. (2022) and Tembe *et al*. (2023). Previous workers had attributed variation in the MCV values in scavenging birds to breed, hormonal and nutritional deficiencies (Jubril *et al*., 2021). However, the finding of this study shows that infection of haemoparasites did not affect the value of the MCV which is similar to the reports of Ogbaje *et al*. (2019) and Boonhoh *et al*. (2023). However, MCV values were higher in infected chickens, possibly reflecting intravascular haemolysis and leading to normocytic normochromic anemia (Lashari *et al*., 2018; Ogbaje *et al*., 2019).

MCHC values were slightly above normal, with higher values in infected chickens ($p < 0.05$), consistent with Olayemi and Arowolo (2009). This may result from haemoconcentration, though seasonal variations could also play a role (Hill *et al*., 2017). The elevated WBC levels in infected chickens suggest an immune response, aligning with Townsend *et al*. (2018), Ogbaje *et al*. (2019), and Jubril *et al*. (2021).

Increased heterophil, lymphocyte, monocyte, and eosinophil counts in infected chickens support the immune response role of these cells (Samani *et al*., 2016; Jubril *et al*., 2021; Wiegmann *et al*., 2021). Although heterophil counts did not show statistical significance, they hint at a potential decrease in resistance to other infections (Umar *et al*., 2022; Wang *et al*., 2023).

The impact of haemoparasites on serum biochemistry parameters, including glucose, total plasma protein, and ALT levels, reflects physiological disturbances. Reduced glucose levels may result from increased metabolic demands and impaired glucose utilization (Sahraei, 2014; Alders *et al*., 2018).Significant decreases in total plasma protein and serum protein in chickens infected with *Plasmodium* suggest severe protein metabolism issues, likely due to liver damage (Tostes *et al*., 2015). Conversely, higher total plasma protein levels in chickens infected with *Haemoproteus* and

Leucocytozoon could indicate an inflammatory response (Wamboi *et al*., 2020).

The lower serum albumin levels in chickens infected with *Plasmodium* and mixed infections suggest potential liver damage or a catabolic state, while higher levels in *Haemoproteus*-infected chickens might reflect a less severe liver involvement. The reduction in serum globulin levels across infected chickens indicates a compromised immune response (Fernández-Cruz *et al*., 2009; Agina *et al*., 2021; Akoolo *et al*., 2022). Reduced ALT levels in *Plasmodium*-infected chickens suggest hepatic dysfunction, though variations in ALT levels across different infections need further investigation (Chughlay *et al*., 2020; Ehiem *et al*., 2021).The observed changes in serum biochemistry parameters in the present study could partly be attributed to the poor nutritional status of the village chickens, as indicated by the management system used to raise them during the study period. These findings are consistent with several studies that have examined variations in biochemistry parameters in chickens under different management systems (Kalita *et al*., 2018; Alam *et al*., 2020; Kraus *et al*., 2021). Malnutrition can worsen the effects of haemoparasite infections by weakening the immune system and diminishing the body's capacity to repair and maintain tissues, as reported by Morales *et al*. (2023). Specifically, protein-energy malnutrition may account for the observed lower levels of total plasma protein, serum albumin, and serum protein in both haemoparasite-infected and uninfected chickens when compared to the reference values for these biochemical parameters in the current study.

The lower ALT levels observed in chickens infected with *Plasmodium* and mixed infections might indicate hepatic dysfunction, although there is a paucity of published work for comparison. However, other studies have shown that ALT is a liver enzyme, and a reduction in its levels indicates hepatocellular injury (Chughlay *et al*., 2020; Ehiem *et al*., 2021). The lack of significant changes in ALT levels in chickens infected with *Haemoproteus* and *Leucocytozoon* suggests that these parasites may cause less hepatic damage or that the liver injury is not severe enough to significantly alter enzyme levels. This finding contrasts with the results of Wamboi *et al*. (2020), who reported a significant decrease in ALT levels in *Haemoproteus*-infected chickens compared to those infected with other hemoparasites and uninfected chickens.

Conclusion

It was concluded that haemoparasites, including *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, are prevalent in village chickens in Gombe State, with an overall prevalence of 19.6% (95% CI = 17.8 – 21.5%). Among these, *Plasmodium* was the most common, affecting 13.9% of the chickens. The infections with the various haemoparasites was found to cause significant alterations in some haematological parameters, resulting in normocytic normochromic anaemia. Additionally, the study indicated that avian haemoparasite infections significantly impact certain serum biochemistry parameters, such as blood glucose, total plasma protein, total serum albumin, total serum protein, total serum globulin, and serum alanine aminotransferase (ALT) levels.

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