*Original Article*

**Sahel Journal of Veterinary Sciences**

*Sahel J. Vet. Sci.* Vol. 18, No. 1, pp 21-26 (2021)


**Article History**
**Received: 19-07-2020**
**Revised: 23-03-2021**
**Accepted: 04-03-2021**
**Published: 05-03-2021**

# Haematological Profile of Naturally Infected Haemoparasite Positive and Negative Japanese Quails (*Coturnix coturnix japonica)*

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## ABSTRACT

Commercial quail farming is economically viable and feasible because quails are resistant to various diseases. But despite this reported resistance, little is known about its resistance to haemoparasites. This study evaluates the haematological changes and haemoparasitic infection of commercially raised quails. Fifty-seven adult quails raised on deep litter were randomly selected for blood sampling in this study. Two milliliters of blood was collected aseptically for complete blood count while blood smears were used for the determination of haemoparasite morphological characteristics. Haemoproteus spp. Plasmodium gallinaceum, and Leucocytozoon spp. were identified in this study. 38 (67%) of the quails were positive for single or mixed infection, 29 (51%) were positive for single infection and 9(16%) for mixed infection. There was a ($P<0.05$) decrease in PCV, Hb, and RBC counts, and an increase in TWBC and eosinophil count in birds with haemoparasite as compared to the uninfected birds. There was ($P<0.05$) increase in total white blood cell and heterophil count in the plasmodium positive birds. Also, total white blood cell, heterophil, lymphocyte and eosinophil count were ($P<0.05$) increased in the Leucocytozoon positive birds. This increase was also observed in birds with mixed infection. The high incidence of haemoparasitic infection in apparently healthy quail, with significant haematological indices deviated from normal, is consistent with reports of the resistance of quail to various disease diseases which thus includes haemoparasitic infection.

**Keywords**: Blood smears; Complete Blood Count; Haemoparasites; Quail

## INTRODUCTION

Poultry production as an aspect of livestock production is important to the biological needs, economic and social development of the people in any nation (Ogunniyi *et al*., 2012). In Nigeria, it serves as a source of income, employment, nutrition (meat and egg) and contributes about 25% of Nigeria non- oil Gross Domestic Product (GDP) earning from the livestock sector (CBN, 2013). Several species of birds are of economic importance, mostly as sources of food, including domestic fowl, pheasant, turkeys, quails, doves, partridges, geese, guinea fowls and woodcocks (Clements, 2007; Bakoji *et al*., 2013). However, this industry is faced with several challenges: high cost of feed, low start up capitals, diseases, poor quality feed and chicks (Adeyemo and Onikoyi, 2012).

Quail is a small, stocky bird with short legs and varied plumage. It's a member of the Phasianidae family (including pheasants and partridges), consisting of several species. The Common Quail (*Coturnix coturnix)* is the wild variety, measuring 16-18 cm and weighing 70-135g (Onyewuchi *et al*., 2013). The Japanese quail (*Coturnix japonica*), domesticated more than 700 years ago, and is the most frequently farmed species for egg and/or meat purpose. It also has low feed requirements, rapid growth, short generation, and gestation periods. It is regarded as a cheap source of animal protein and is more resistant to diseases than chicken and other poultry (Jatoi *et al*., 2013).

Quail farming in Nigeria is profitable due to the high demand for quail eggs and meat (Owen and Dike, 2013). The return on investment in quail farming in Nigeria is fast and relatively high, it, therefore, plays a very important role in the food security, health, wealth and employment creation (Owen and Dike, 2013). Despite the reported resistance of quail to diseases, there is a gap in the knowledge that exists regarding its resistance to haemoparasites.

Avian haemoparasite infection is associated with clinical signs such as anorexia, depression, anaemia, emaciation, reduced productivity and high mortalities (Sol *et al*., 2003; Dun *et al*., 2013). Commonly reported haemoparasites of avian species include; *Haemoproteus sp*, *Plasmodium sp*, *Leucocytozoon*, *Hepatozoon sp, Aegyptionnella sp* and nematode microfilariae (Olayemi *et al*., 2014). Parasites in the subgenera *Haemoproteus* are transmitted by hippoboscid s while those in the subgenera *Parahaemoproteus* are trans mitted by *Culicoides* spp. (Pacheco *et al*., 2018). These blood parasites can exert important selection pressure on their hosts through effects on survival and reproduction **(**Mavuti, 2010)**.**

Haematological parameters are good indicators of the physiological status of animals. Studying the numbers and morphology of the cellular elements of the blood; red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) is useful in the monitoring and diagnosis of disease (Merck Manual, 2012; Isaac *et al*., 2013). Hence, this study is aimed at evaluating the incidence of haemoparasites in commercial quails and their associated haematology changes.

## MATERIALS AND METHODS

### Experimental Birds and Sample Collection

Fifty-seven adult quails reared on deep litter system, fed commercial feed with water provided *ad libitum* raised for commercial purposes from the National Veterinary Research Institute, Ikire, Osun State quail farm were used for this study. Two millilitres of blood was collected aseptically from the jugular vein using a 23 gauge sterile hypodermic needle from the cephalic vein and dispensed into lithium heparin bottles for haematological analysis.

### Blood Smear Preparation

Smears were prepared from the blood sample using a clean glass slide. A drop of blood was put on the slide and spread with another glass slide held at an angle 40 to 45° and push forward firmly. The smears were properly dried and fixed using 5% methanol while lying flat on the table. The pre-dried slides were set down in a slanted position on their narrow edge with the film side down for about 3 minutes and allowed to air-dry properly. The properly air-dried smears were then transferred to the staining trough and properly arranged in it to facilitate proper staining as described by Cheesbrough (2000). Giemsa stain was added to the smear in the trough for about 10 minutes. The stain was washed off and differentiated in buffered distilled water pH 7.2. The smears were then slanted to allow the excess water to drip off and to aid drying of the smear.

### Blood Smear Examination

Microscopic examination of Giemsa-stained slide for the presence of haemoparasites was done using an oil immersion lens (Holmstad *et al*., 2003). Using a high dry objective lens, oil immersion (×100) of the light microscope, the stained smears were observed for parasites and the identified based on their morphological characteristics (Valkiûnas *et al*., 2005).

### Haematology

Quantitative and qualitative analysis of haematological indices were carried out by standard methods (Weiss and Wardrop 2010). Red Blood Cell Count was carried out by haematocytometer method. Packed Cell Volume (PCV) was determined using the microhaematocrit method. Determination of haemoglobin concentration was done using the Cyanmethaemoglobin method while platelet Count was carried out using the Direct Method. Total White Blood Cell Count (TWBC) was determined using improved Hawksley haemocytometer while differential leucocyte counts were done using Wright-Giemsa-stained blood smears made from the collected uncoagulated blood and examined under the light microscope using oil immersion objective.

### Statistical Analysis

All data were expressed as Means ± Standard Error of Means (S.E.M). The incidence of the haemoparasites was calculated in percentage for the categorization of the haemoparasite positive and haemoparasite negative group. Student t-test was used to compare the mean between these groups group while the comparison of means of more than three groups was analysed using one way ANOVA. SPSS version 20 software package was used for this analysis and values of $P<0.05$ was considered statistically significant.

### Ethical Statement

The protocol for this research was approved by the Animal Care and Use Research Ethics Committee, University of Ibadan, Ibadan, Oyo State, Nigeria.

## RESULTS

### Blood Parasite Incidence and Morphology

Haemoparasites identified in this study are *Haemoproteus* spp*, Plasmodium* spp, and *Leucocytozoon* spp. 38 (67%) of the quails were positive for single or mixed infection of haemoparasites, 29 (51%) were positive for single infection and 9(16%) for mixed infection. *Haemoproteus* spp. had the highest occurrence of 32%, while the least was 9% for *Leucocytozoon* spp.

The gametocytes of *Haemoproteus* spp having an elongated appearance in red blood cell is presented in (Figure 1). *Plasmodium* spp trophozoite distorting the morphology of red blood cell (Figure 2) and gametocyte of *Leucocytozoon* spp which is spherical to ovoid, and enlarged, distorts the infected cell producing a football-like appearance in a mononuclear cell (Figure 3).

**Figure 1:** Gametocytes of *Haemoproteus spp.* appears elongated (arrow) in quail red blood cell. (Giemsa, x1000).

**Haematology**

The parasitaemia observed in this study resulted in a significant decrease of red cell indices but there was no clinically apparent anaemia.

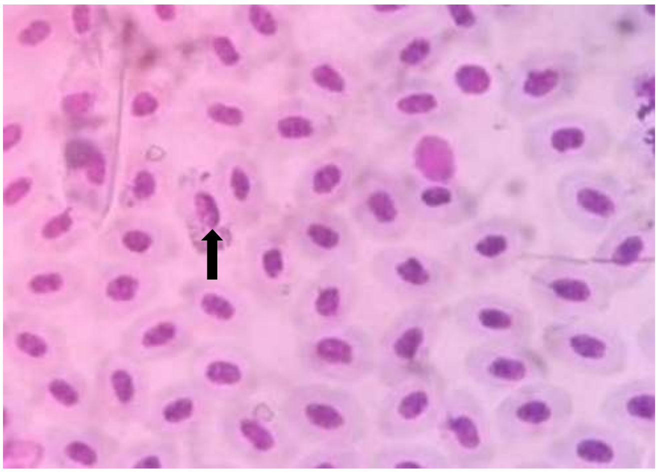

**Figure 2:** Trophozoite of *Plasmodium elongatum* (arrow) in quail red blood cell cytoplasm (Giemsa stain x1000).

**Figure 3:** Gametocytes of *Leucocytozoon spp*, spherical to ovoid, distorting the infected cell producing a football-like appearance (arrow) in a mononuclear cell of quail, (Giemsa, x1000).

The haematological parameter in haemoparasite positive and negative quails showed (P<0.05) decrease in PCV, HB and a (P<0.05) increase in TWBC, heterophils and eosinophil counts (Table 1).

There was a (P<0.05) decrease in the PCV of Haemoproteus and Leucocytozoon infected quail and a (P<0.05) decrease in Hb and RBC counts of quails infected with Leucocytozoon. Quails infected with *Haemoproteu*s spp had (P<0.05) increased TWBC and eosinophil counts, while *Leucocytozoon* infected quails had a (P<0.05) increase in TWBC, heterophil, lymphocyte and eosinophil counts. However, quails infected with *Plasmodium* spp had (P<0.05) increase in heterophil count only (Table 2). Although there was a decrease in haematological parameters in the haemoparasite infected quails as compared to the uninfected group, there was a (P<0.05) decrease in PCV and HB in birds infected with both *Haemoproteus and leucocytozoon* as compared to quails infected with *Haemoproteus and Plasmodium s*pecies. Furthermore, quails infected with *Haemoproteus and Leucocytozoon* had a (P<0.05) increase in TWBC heterophils, lymphocyte and eosinophils counts as compared with the *Haemoproteus* and *Plasmodium* infected quails that had (P<0.05) increase in TWBC values alone (Table 3).

**DISCUSSION**

The presence of haemoparasites and haematological response as a biomarker of infection might give an insight into the resistance of quail to haemoparasites. In this study, *Haemoproteus spp*, *Plasmodium spp* and *Leucozytozoon spp* in single and mixed infections were observed. This report is consistent with that of (Rawaa, 2012) in quails and (Karamba *et al.,* 2012) in chicken and other birds. The high load of blood parasites (79%) is comparable to the studies done by (Valkiûnas *et al.,* 2005) who reported the prevalence of avian blood parasites in Uganda to be 61.9%, while (Njunga, 2003) in Malawi found the prevalence of haemoparasites in chicken to be 71%.

**Table 1**: Mean (±SD) of haematological parameters in haemoparasite positive and negative quails.

| Haematological Parameters | Haemoparasite negative (n = 19) | Haemoparasite positive (n=38) |
|---|---|---|
| **PCV (%)** | 43.50 ± 7.07 | 37.83 ± 7.07** |
| **Hb (g/dL)** | 14.4 ± 2.2 | 12.6 ± 2.1** |
| **RBC (×10$^6$/ µL)** | 4.02 ± 0.44 | 3.75 ± 0.71 |
| **Platelet (×10$^5$/ µL)** | 203 ± 67 | 222 ± 11 |
| **Total WBC (×10$^3$/ µL)** | 16.94 ± 4.64 | 18.94 ± 5.70** |
| **Heterophil (×10$^3$/ µL)** | 6.65 ± 4.74 | 7.85 ± 4.74** |
| **Lymphocyte (×10$^3$/µL)** | 9.36 ± 2.92 | 10.46 ± 2.75 |
| **Monocyte (×10$^3$/ µL)** | 0.22 ± 0.28 | 0.25 ± 0.29 |
| **Eosinophil (×10$^3$/ µL)** | 0.69 ± 0.36 | 1.13 ± 0.43** |
| **Basophil (×10$^3$/ µL)** | 0.06 ± 0.02 | 0.08 ± 0.01 |

Standard Deviation (SD), Pack Cell Volume (PCV), Haemoglobin (HB), Red Blood Cell (RBC), White Blood Cell (WBC), Asterisk (*): Statistically significant at p<0.05.

Distribution of infection with haemoparasite depends upon the environment and different exposure of birds to vectors; the exposure may depend on the time of daily activities of birds, selected place of nesting, sampling effort and location (Sabuni *et al.*, 2011). The different parasite identified could be due to differences in habitat, climate and specie affected (Lambin *et al*., 2010).

The occurrence of mixed infection also agrees with a previous report (Sadiq *et al*., 2003) that identified the same three haemoparasites (*Plasmodium* spp, *Leucocytozoon* spp and *Haemoproteus* spp) though in chickens. This finding is contrary to the findings of (Peninah *et al*., 2020) in Kenya, who reported the occurrence of *Aegyptinella* spp in addition to the other haemoparasites. This could be attributed to species differences and the geographical location of the study area.

**Table 2**: Mean (±SD) of haematological indices in *Haemoproteus, Plasmodium, Leucocytozoon* infected and non-infected quail.

| | Haemoparasites | | | |
|---|---|---|---|---|
| **Haematological Indices** | Non-infected n=19 | Haemoproteus n=18 | Plasmodium n=6 | Leucocytozoon n=5 |
| **PCV (%)** | 43.50 ± 7.07 | 37.36 ± 6.40** | 38.33 ±10.57 | 31.80 ± 10.11*** |
| **Hb (g/dL)** | 14.42 ± 2.25 | 11.60 ± 1.7 | 12.76 ± 3.33 | 10.70 ± 3.18** |
| **RBC (×10⁶/ µL)** | 4.02 ± 0.44 | 3.13 ± 0.5 | 3.92 ± 1.09 | 3.21 ± 0.93* |
| **Platelet (×10⁵/ µL)** | 203 ± 67 | 215 ± 12 | 222 ± 11 | 200 ± 27 |
| **Total WBC (×10³/µL)** | 16.94 ± 4.64 | 18.91 ± 3.10* | 17.41 ± 7.18 | 22.59 ± 5.03** |
| **Heterophil (×10³/ µL)** | 6.65 ± 4.74 | 7.15 ± 5.13 | 7.04 ±5.62** | 8.24 ± 5.50** |
| **Lymphocyte(×10³/µL)** | 9.36 ± 2.92 | 10.52 ± 4.36 | 9.42 ± 2.72 | 12.23 ± 2.36* |
| **Monocyte (×10³/ µL)** | 0.22 ± 0.28 | 0.38 ± 0.30 | 0.39 ± 0.40 | 0.36 ± 0.19 |
| **Eosinophil (×10³/ µL)** | 0.69 ± 0.36 | 1.16 ± 0.39** | 0.72 ± 0.40 | 1.38 ± 0.34** |
| **Basophil (×10³/ µL)** | 0.06 ± 0.02 | 0.07 ± 0.04 | 0.06 ± 0.10 | 0.073 ± 0.10 |

Standard Deviation (SD), Pack Cell Volume (PCV), Haemoglobin (HB), Red Blood Cell (RBC), White Blood Cell (WBC), Asterisk (*): Statistically significant at $p<0.05$.

**Table 3**: Mean (±SD) value of erythrocyte indices in quails with mixed infection and the non-infected group.

| Haematological Parameters | Non-infected (n=19) | Haemoproteus/ Plasmodium (n=5) | Haemoproteus/ Leucocytozoon (n= 4) |
|---|---|---|---|
| **PCV (%)** | 42.97 ± 7.04 | 38.10 ± 4.30 | 35.27 ± 7.39** |
| **Hb (g/dL)** | 14.25 ± 2.23 | 11.80 ± 0.62 | 10.93 ± 1.54* |
| **RBC (×10⁶/ µL)** | 4.02 ± 0.45 | 3.11 ± 0.93 | 3.28 ± 0.73 |
| **Platelet (×10⁵/ µL)** | 203 ± 67 | 212 ± 10 | 226 ± 11 |
| **Total WBC (×10³/µL)** | 16.94 ± 4.64 | 19.01 ± 6.09* | 20.01 ± 3.91** |
| **Heterophil (×10³/ µL)** | 6.65 ± 4.74 | 5.65 ± 5.36 | 8.42 ± 5.16* |
| **Lymphocyte(×10³/µL)** | 9.36 ± 2.92 | 9.96 ± 2.92 | 11.71 ± 2.73* |
| **Monocyte (×10³/ µL)** | 0.22 ± 0.28 | 0.32 ± 0.28 | 0.39 ± 0.30 |
| **Eosinophil (×10³/ µL)** | 0.69 ± 0.36 | 0.69 ± 0.36 | 1.28 ± 0.44** |
| **Basophil (×10³/ µL)** | 0.06 ± 0.02 | 0.06 ± 0.08 | 0.08 ± 0.01 |

Standard Deviation (SD), Pack Cell Volume (PCV), Haemoglobin (HB), Red Blood Cell (RBC), White Blood Cell (WBC), Asterisk (*): Statistically significant at $p<0.05$.

In this study, *Haemoproteus* spp. had the highest prevalence of 32%, while the least was reported for Leucocytozoon spp. (9%). This finding is consistent with the studies by (Cerny *et al*., 2011; Karamba *et al.,* 2012) but contrary to the findings by studies by (Sabuni *et al*., 2011) who indicated *Haemoproteus* spp as the least and this may be attributed to specie difference as it was observed in chickens.

In this study, the sampled quail harboured these parasites without any obvious clinical signs. The significant haematological changes without clinical anaemia and leucocytosis, suggests that despite the parasitaemia in the bird, they were not clinically sick due to the absence of significant deviation of clinical values from normal. This finding is consistent with the studies of (Potti, 2007; Astudillo *et al*., 2013) who reported that the changes in haematological values, particularly the PCV could be varying and related to other factors such as metabolism, workload or genetics.

The infected but not clinically sick quails could harbour the parasite for a lifetime, therefore serving as reservoirs to the parasites. The parasites could be transmitted by vectors or through contact with other poultry in the sample facilities as have been reported by (Garba, 2008).

From the three haemoparasites encountered, *Plasmodium gallinaceum* is the most pathogenic, as reported in chicken (Argilla *et al*., 2013), but it differed in this study as Leucocytozoon infected bird showed more deviation from normal values. Decreased PCV, Hb and an increased WBC, heterophils, lymphocyte and eosinophil count were more apparent in the quails infested with leucocytozoon as well as in mixed infection with *Haemoproteus*, compared with the quails infested with plasmodium and Haemoproteus. The increase in WBC indicates body response to the presence of the parasite especially with increased eosinophil count. Haemoproteus are responsible for some instances of mortality in birds. Due to the application of molecular diagnostic techniques, the reports about this specie being harmless except in chronic cases is on-going and needs reconsideration as the parasites worth more attention to better understand their role in quail parasitism (Valkiūnas, 2015). Levin *et al*., 2013 reported that the number of *H. lophortyx* blood stages is highly correlated with flock mortality, and most quail that die during an outbreak are in good body condition, suggesting that haemolysis is the cause of death in parasitized birds.

The statistically significant increase in WBC and eosinophil count in the haemoparasite positive group and the ($P<0.05$) increase in WBC indicates a response to the infection. This is consistent with the report by (Cotter, 2014) who reported leucocytosis as an indicator of stress and or infection.

There was a significant increase in heterophil count in the plasmodium positive group and a significant increase in WBC, heterophils count, Lymphocyte count and eosinophil count in the *Leucocytozoon* positive group and in mixed infection with *Leucocytozoon* indicating that quails show more response to *Leucocytozoon* and *Plasmodium* than *Haemoproteus*. The high white blood cell counts may be an indicator of parasitism, nutritional and environmental factors that can trigger leukocytosis (Cotter, 2014).

**Conclusion**

This study revealed that there is a high occurrence of haemoparasitic infection in apparently healthy quails with a significant deviation of haematological parameters from normal values but with no apparent clinical signs. And this is consistent with the reports of resistance of quails to diseases. However, there is a need for further studies to assess the severity of the exiting parasitaemia and associated haematological changes.

**Acknowledgements**

The authors wish to acknowledge the National Veterinary Research Institute (NVRI), unit in Osun State for their support towards this research.

**Conflict of Interest**

The authors have no conflict of interest to declare.

**Authors Contribution**

RA designed, supervised and reviewed manuscript. AJJ and OG carried out laboratory analysis and data interpretation. AAA and LAA prepared the draft manuscript and participated in data analysis.

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