Study of Some Haematological Parameters of Japanese Quail (*Coturnix coturnix japonica*) Experimentally Infected with *Salmonella enterica* Serovar Gallinarum

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors IJB, JOOB, SBO and MYF designed the study, wrote the protocol and interpreted the data. Authors PRK, AGR and IST anchored the field study, gathered the initial data and performed preliminary data analysis. While authors GDM, JSA and PAO managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine some haematological changes in Japanese quails (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar Gallinarum

Methodology: A total of 160 (108 males and 52 females) Japanese quails (*Coturnix coturnix japonica*) were used in this study. The quails were obtained at the age of four weeks from the Poultry Division of the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They were randomly selected and assigned into four groups (A, B, C and D) of forty quails each. Groups A, B and C were infected with *Salmonella enterica* serovar Gallinarum per os at the dose of $10^6$, $10^4$ and $10^2$, respectively, while group D served as the control. Blood with anticoagulant

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1. INTRODUCTION

The genus *Salmonella* is a member of the Family *Enterobacteriaceae* and consists of Gram-negative, non-spore forming bacilli [1]. *Salmonella enterica* serovar Gallinarum (*Salmonella Gallinarum*) is a non-motile host adaptive *Salmonella* that causes fowl typhoid (FT), a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and pathological lesions [2]. With the continuous expansion of poultry farming in Nigeria, FT has gained ground as a major disease of poultry which can cause heavy economic losses through mortality and pathological lesions [3]. The impact of FT is hard to judge in developing countries due to lack of systematic surveillance, but its importance is clear, from both published and anecdotal evidence, with outbreaks in Mexico, Argentina, Nigeria and India [4]. Endemic FT is still found in many countries in both commercial production and backyard flocks including countries with expanding poultry industries such as Brazil and South Korea where there has been considerable research activity in recent years [4]. Quails are ideally suited for avian research, because of their small size and require little cage space for rearing. They are easy to raise and are suitable for genetic studies since they rapidly attain sexual maturity [5]. Quails are hardy birds which thrive very well in cages and are relatively inexpensive to maintain. They are birds that households can keep without stress [6]. The Japanese quail has the potential of filling some of the gap in the protein needs of Nigerians. It is therefore important to investigate diseases that can interfere with quail production such as FT in order to control them [7]. Quail meat and eggs are known for high quality protein, high biological value and low caloric content, thus making them good choices for hypertension prone individuals [8]. Because its lifespan is relatively short and its physiology is comparable to that of humans, the adult quail is useful for studies of diseases and aging [6]. An increasing number and variety of quails are being kept for food, experimental use, released on hunting preserves, preservation of endangered species, zoological displays and as companion birds. This is because they are easy to raise and are suitable for genetic studies since they rapidly attain sexual maturity [9].

This study presents changes in some haematological parameters in Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar Gallinarum (*Salmonella Gallinarum*) reared in Zaria, Northern, Nigeria.

2. METHODOLOGY

2.1 Area of Study

The study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7° and 11°N, and longitude 7° and 44°E. The average rainfall of this zone ranges from 1,000 to 1,250 mm annually, and the average temperature ranges from 17°C to 33°C [10].

2.2 Experimental Birds

A total of 160, four-week old Japanese quails (108 males and 52 females) were obtained from

(ethylenediamine tetra-acetic acid) was used for the determination of haematological parameters. **Results:** There were no significant differences in haematological parameters (P > 0.05) between the groups before infection. There were, however, significant changes (P<0.05) in haematological parameters such as red blood cells (RBC), haemoglobin (Hb) concentrations, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), heterophil and lymphocyte counts between the infected groups when compared with the control group. There were also significant changes (P<0.05) in these haematological parameters post-infection compared to before infection within each infected group.

**Conclusion:** This study has shown that *Salmonella gallinarum* causes decrease in some haematological parameters, such as packed cell volume (10%) and red blood cell count (10%) and haemoglobin concentration (5%) and increase in haematological indices, such as mean corpuscular volume (5%), mean corpuscular haemoglobin (30%), and mean corpuscular haemoglobin concentration (30%) above control group values in Japanese quails (*Coturnix coturnix japonica*). These findings could be useful in the diagnosis of subclinical cases of fowl typhoid in the Japanese quails.

**Keywords:** Haematology; quail; experimental; *Salmonella*.
the Poultry Division of National Veterinary Research Institute, Vom. The quails were randomly selected and divided into four groups (A, B, C and D) of 40 birds each; with 27 males and 13 females in group A, 23 males and 17 females in group B, 29 males and 11 females in group C as well as group D.

2.3 Housing and Feeding

The quails were kept in deep litter but separated into different compartments using wire mesh measuring 120 × 140 × 120 cm in size in an enclosed house with the litter changed every week throughout the experimental period. The control group was kept in a separate room 20meters away from the infected groups. The quails were allowed to acclimatize for two weeks, during which they were observed for clinical signs of any infection. During the acclimation period, the quails were fed on commercial chick mash, and thereafter switched to commercial layer mash throughout the period of the research which lasted 120 days. They were fed and provided with water ad libitum throughout the study period.

2.4 Bacteriological Monitoring Before Infection

Before infection, cloacal swabs were collected from all the quails using sterile swabs in order to confirm if they were free from Salmonella organism or indeed any other bacterial pathogens. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) and blood agar (BA) using standard laboratory methods [2].

2.5 Source of Organism and Infectivity Procedure

The bacterium, Salmonella enterica serovar Gallinarum, was obtained from the bacterial culture bank of the Central Diagnostic Laboratory, National Veterinary Research Institute, Vom. The isolate was from day old chick that died of natural Salmonella Gallinarum infection. The lyophilized bacterium from the culture bank was reactivated by sub-culturing on blood agar (BA) and MacConkey agar (MCA). The resulting colonies were examined for their features, colour and morphology and tested for Gram-reaction (Gram-negative). Three colonies were scooped and inoculated into 20 ml of nutrient broth and this was incubated for 24 hours at 37°C after which a ten-fold dilution was carried out in test tubes. The colony counts from the test tubes were determined. To obtain the number of organisms that were inoculated into the quails, the number of organisms was multiplied by volume by the dilution factor (CFU= No. of colony × Volume × Reciprocal of Dilution factor) [11]. Challenge of the quails was done orally. Group A quails received a dose of 1 × 10^6 organisms/0.2 ml of nutrient broth, group B quails received a dose of 1 × 10^5 organisms/0.2 ml of nutrient broth, while group C quails received a dose of 1 × 10^4 organisms/0.2 ml of nutrient broth. Group D quails served as control and were not challenged with the bacterium, but were given bacteria-free nutrient broth in accordance with the method of Miles and Misra [11].

2.6 Determination of Haematological Parameters

The baseline values for haematological parameters were determined before the commencement of the experiment, according to standard procedures [12-14]. Blood samples (2 ml) were collected via the wing vein, using 25 gauge sterile hypodermic needles and syringes. These were collected 5 days before challenge and 5 days after challenge, so as to reduce stress pre-infection and to get the blood picture within the incubation period of the disease respectively. Blood for haematological analysis were collected into 2 ml labelled Bijou-bottles, containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Red blood cell count, packed cell volume and haemoglobin concentration were determine using Mindray autohaemo analyser, model BC-3000 PLUS, manufactured by Shenzhen Mindray Biomedical Electronics Ltd, China. The haemo analyser machine was switched on and it automatically flushed the fluidic lines, checked the background and entered the count screen. The menu key was pressed to select the sample mode, and whole blood was selected. The blood in the sample tubes were mixed well using Stuart roller mixer machine model SRT9D, after bringing it to room temperature. It was then presented to the analyser probe tip making sure it submerges well into the sample. The aspirate key was pressed to start the count. The result was printed out in a hard copy automatically from the machine and recorded [13].

2.7 Differential Leukocyte Count

For differential leukocyte count, samples were mixed well using Stuart roller mixer machine
model SRT9D. A drop of blood was placed one centimeter away from the edge of clean, grease-free slide using pipette. A spreader was placed in front of the blood to allow the blood to spread across its edge. The spreader was moved forward quickly and smoothly. The smear was allowed to air dry, and then labelled. The air dried smear was then placed on a staining rack, flooded with filtered Leishman's stain and allowed to fix and stain for two minutes. The stained smears were then flooded with equal volume of buffered distilled water (pH 6.8) and allowed to rinse for 8 minutes. Excess stain was washed off with tap water and allowed to differentiate to salmon pink colour. The slides were air dried and viewed using oil immersion objective, starting from the thin end. A systematic meander system of slow, careful and detailed leucocytes count was made, and the result recorded [14].

2.8 Data Analysis

Data obtained were expressed as mean±S.E.M (standard error of the mean). They were subjected to repeated measure of one way analysis of variance (ANOVA) to determine the difference in the parameters before infection and post-infection between the groups. This was followed by Tukey’s post-hoc multiple comparison tests using Graph-pad prism version 4.0 for windows. Values of P < 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Figs. 1 to 8 show mean haemoglobin concentration (Fig. 1), mean red blood cell (RBC) count (Fig. 2), mean packed cell volume (PCV) (Fig. 3), mean corpuscular volume (MCV) (Fig. 4), mean corpuscular haemoglobin (MCH) (Fig. 5), mean corpuscular haemoglobin concentration (MCHC) (Fig. 6), mean heterophil count (Fig. 7) and mean lymphocyte count (Fig. 8), respectively before and after infection. The haematological changes in the quails presented slight reduction in mean haemoglobin concentration (Fig. 1), mean red blood cell (RBC) count (Fig. 2) and mean packed cell volume (PCV) (Fig. 3) in all the infected groups. The reduction observed in this study corresponded with the acute phase of fowl typhoid in which anemia has been reported in chickens [15,16] Assoku and Penhale [15] suggested that the anemia associated with acute fowl typhoid in chicken may be a direct result of the increased ability of the reticuloendothelial cells to take up erythrocytes. Christensen et al. [17] indicated that the modification of the erythrocytes is associated directly (lipopolysaccharide/outer membrane proteins) or indirectly (by induction of antibodies) or both, to the number of bacteria present in the tissues. There was no significant change (p>0.05) in the mean corpuscular volume (MCV) post-infection (Fig. 4), but there were significant changes (p<0.05) in the mean corpuscular haemoglobin (MCH) (Fig. 5) and mean corpuscular haemoglobin concentration (MCHC) (Fig. 6) in the infected groups, when compared with the pre-infection values and with the corresponding mean values obtained in the control group. This haematological result could be due to haemorrhages and congestion observed in some organs in this study, which is in agreement with findings of Shah et al. [18] in broiler chickens.
Fig. 3. Mean packed cell volume (PCV) before and after infection of quails with *Salmonella enterica* serovar Gallinarum

Fig. 4. Mean corpuscular volume before and after infection of quails with *Salmonella enterica* serovar Gallinarum

Fig. 5. Mean corpuscular haemoglobin before and after infection of quails with *Salmonella enterica* serovar Gallinarum

Fig. 6. Mean corpuscular haemoglobin concentration before and after infection of quails with *Salmonella enterica* serovar Gallinarum

Fig. 7. Mean heterophil count before and after infection of quails with *Salmonella enterica* serovar Gallinarum

Fig. 8. Mean lymphocyte count before and after infection of quails with *Salmonella enterica* serovar Gallinarum
The differential leukocyte counts revealed a slight increase (p<0.05) in percentage of heterophils (Fig. 7) in all the infected groups and significant decrease (p<0.05) in the relative percentage of lymphocytes (Fig. 8) in infected group A, but slight increase (P>0.05) in infected group C and no changes observed in infected group B and control group D (p>0.05). This result is slightly different from the report of Shah et al. [18] who reported that leukocytosis in broiler chicken infected with Salmonella Gallinarum was due to increase in both the number of heterophils and lymphocytes. This difference could be due to species variation in response to infection.

4. CONCLUSION

This study has shown that Salmonella Gallinarum causes decrease in some haematological parameters, such as packed cell volume (10%) and red blood cell count (10%) and haemoglobin concentration (5%) and increase in haematological indices, such as mean corpuscular volume (5%), mean corpuscular haemoglobin (30%), and mean corpuscular haemoglobin concentration (30%) above control group values in Japanese quails (Coturnix coturnix japonica). These findings could be useful in the diagnosis of subclinical cases of fowl typhoid in the Japanese quails.

5. RECOMMENDATIONS

Further studies should be carried out to find out appropriate fowl typhoid vaccination schedule for the Japanese quails. Japanese quails reared in Nigeria should be vaccinated as a preventive measure against fowl typhoid. Those keeping quails should adhere to strict biosecurity measures as means of prevention and control of fowl typhoid in the quail farm. Additional studies to further characterize the hematological parameters of quails with systemic infection should be carried out. Further studies should be carried out in the areas of pathogenesis and virulence of other organisms that were previously thought not to affect quails.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Popoff MY, Bockemuhl J, Gheeshing LL. Antigenic formulas of the Salmonella Serovars, 8th revision. WHO Collaborating Centre for Reference and Research on Salmonella. Supplement 2001 (No: 45) to the Kauffmann-White Scheme Research in Microbiology. 2003;154:173-174.
2. Parmer D, Davies R. Fowl typhoid in small backyard laying flock. The Veterinary Record. 2007;160:348.
3. Agbaje M, Davies R, Oyekunle MA, Ojo OE, Fasina FO, Akinduti PA. Observation on the occurrence and transmission pattern of Salmonella gallinarum in commercial poultry farms in Ogun State, South Western Nigeria. African Journal of Microbiology Research. 2010;4(9):796-800.
4. Barrow PA, Jones MA, Smith AL, Wigley P. The long view: Salmonella - the last forty years. Avian Pathology. 2012;41(4):413-420.
5. Haruna ES, Musa U, Lombin LH, Tat PB, Shamaki PD, Okewale PA, Molokwu JU. Introduction of quail production in Nigeria. Nigerian Veterinary Journal. 1997;18:104-107.
6. Huss D, Poynter G, Lansford R. Japanese quail (Coturnix coturnix japonica) as a laboratory animal model. Department of Biology, Biological Imaging Centre, California Institute of Technology, Pasadena, CA, USA. Laboratory Animal (NY). 2008;37(11):513-519.
7. National Veterinary Research Institute Quail production in the tropics: National Veterinary Research Institute, Vom, Nigeria. 2008;75-92.
8. Chindo HJ, Olowoniyan FO. Processing and utilization of quail products. A paper presented at national workshop on quail production for sustainable household – protein intake. National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria. 2006;69–74.
9. Khare MC, Grun J, Adams EU. In: Marek’s disease in Japanese quail - A pathological, virological and serological study. Poultry Science. 1975;54:2066-2081.
10. Saidu L, Abdu PA, Umoh JU, Abdullahi US. Diseases of indigenous chicken. Bulletin Animal Health Production in Africa. 1994;42:19–23.
11. Miles AA, Misra SA. The estimation of bacterial power of the blood. Journal of Hygiene. 1938;38:732.
haemoglobin, haemagglutination inhibition antibody titre and total protein of Japanese quails (*Coturnix coturnix japonica*) administered different doses of Newcastle disease virus. Journal of Animal and Veterinary Advances. 2008;7(4):418-424.

13. Freitas-Neto OC, Arroyave W, Alessi AC, Fagliari JJ, Berchieri Jr A. Infection of commercial laying hens with *Salmonella gallinarum*: Clinical, anatomopathological and haematological studies. Brazilian Journal of Poultry Science. 2007;9(2):133-141.

14. Beyaz L, Atasever A, Aydin F, Gumusoy KS, Abay S. Pathological and clinical findings and tissue distribution of *Salmonella gallinarum* infection in turkey pouls. Turkish Journal of Veterinary and Animal Sciences. 2010;34:101-110.

15. Assoku RK, Penhale WJ. The anaemia in fowl typhoid: Immunopathogenesis and associated patterns of erythrocyte destruction. Journal of Comparative Pathology. 1978;88:219-236.

16. Prasanna K, Paliwal OP. Experimental fowl typhoid and pullorum disease in chickens: Clinical and patho-morphological studies. Indian Journal of Veterinary Pathology. 2002;26:528-531.

17. Christensen JP, Barrow PA, Olsen JE, Poulsen JSD, Bisgaard M. Correlation between viable counts of *Salmonella gallinarum* in spleen and liver and the development of anaemia in chickens as seen in experimental fowl typhoid. Avian Pathology. 1996;25:769-783.

18. Shah S, Kamil S, Darzi M, Mir M, Bhat S. Haematological and some biochemical changes in experimental fowl typhoid infection in broiler chickens. Comparative Clinical Pathology. Academic Journal. 2013;22(1):83.

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