

Gross and Histo-Pathological Changes in Japanese Quail (*Coturnix Coturnix Japonica*) Experimentally Infected with *Salmonella Enterica* Serovar Gallinarum

Barde Israel Joshua^{1,*}, Bale James Olaniyi Olabode², Oladele Sunday Blessing²,
Fatihu Mohammed Yakassai², Kumbish Peterside Rinle¹, Chukwu Doris Isioma¹,
Rimfa Amos Gambo¹, Akanbi Babatunde Olatunde¹, Moses Gyang Davou¹, Ahmed James Saidu¹,
Okewole Philip Ademola¹

¹Department of Central Diagnostic Laboratory, National Veterinary Research Institute, Plateau State, Nigeria

²Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Email address:

israelbarde@yahoo.com (B. I. Joshua)

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Abstract: A total of 160 (108 males and 52 females) Japanese quails (*Coturnix coturnix japonica*) were used for the experiment. The quails were obtained at the age of four weeks from the Poultry Division of the National Veterinary Research Institute (NVRI), 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. Mortality rate ranged from 30% to 35%. Grossly, congestion of the liver, lung and ovarian follicle were generally observed in all the infected groups. Also bronzed liver, hepato-splenomegaly, swollen and congested kidney and ascites were observed. Histopathological lesions included; congestion and cellular infiltration of various organs (liver, lung and heart), as well as hepatic vacuolation and hemorrhages. The high mortality rate, gross and histo-pathological findings in this study demonstrate that Japanese quails (*Coturnix coturnix japonica*) are highly susceptible to *Salmonella enterica* serovar Gallinarum.

Keywords: Gross-Histopathology, Japanese- Quail, *Salmonella* Gallinarum

1. Introduction

The genus *Salmonella* is a member of the Family *Enterobacteriaceae* and consists of Gram-negative, non-spore forming bacilli (Popoff *et al.*, 2003). *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 (Parmer and Davies, 2007). With the continuous expansion of poultry farming in Nigeria, FT has become endemic in poultry resulting in heavy economic losses through mortality and pathological lesions (Agbaje *et al.*, 2010). 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 (Haruna *et al.*, 1997). Quails are hardy birds which thrive very well in cages and are relatively inexpensive to maintain. They are birds that every household can keep without stress (Huss *et al.*, 2008). Although quail farming contributes to the alleviation of protein- deficiency in the diets of people in developing countries, they have largely been neglected as a livestock species due to their small size. Most farmers have focused mainly on chicken production. Thus, the potential contributions of quail to food production have been greatly ignored and or underestimated by policy makers in the agricultural sector in these countries. Nigerians are interested in quail production especially for medicinal reasons and so quail farming should be encouraged with a special focus on the

traditional subsistence rearing system as practiced by resource-limited farmers (NVRI, 2008).

There is a scarcity of information on microscopic lesions of FT in chickens and quails, and most of the lesions described are from field cases which may be complicated by other bacterial and/or viral agents in chickens and to a lesser extent quails (Chadfield *et al.*, 2003). Japanese quails have received less attention relative to other poultry species by researchers (Agrey *et al.*, 2003). The Japanese quail has the potential of filling the gap in the protein needs of Nigerians, it is therefore important to investigate diseases that can interfere with production such as FT in order to determine effective control measures. Quail meat and eggs are known for high quality protein, high biological value and low caloric value, thus making them good choices for hypertension prone individuals (NVRI, 2008).

This study evaluated the gross and histopathological changes in Japanese quail (*Coturnix coturnix japonica*) infected with *Salmonella enterica* serovar Gallinarum (*Salmonella* Gallinarum) in Zaria, Kaduna State, 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 (Saidu *et al.*, 1994).

2.2. Experimental Birds

A total of 160, four-week old Japanese quails (108 males and 52 females) were obtained from the Poultry Division of National Veterinary Research Institute, Vom, Nigeria and used for the experiment. They 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 allowed to acclimatize for two weeks. The quails were kept in mesh cages of 120 × 140 × 120 cm in size in an enclosed house with the litter changed every week throughout the experimental period. The quails were fed for two weeks on commercial chick mash, after which they were fed commercial layer mash throughout the remaining period of the research which lasted 120 days. They were fed and provided with water *ad libitum* during this period.

2.4. Bacteriological Monitoring Before Infection

Before infection, cloacal swabs were collected from all the quails in order to confirm if they were free from *Salmonella*

organism. 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 (Parmer and Davies, 2007).

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, Nigeria. The *Salmonella* isolate was from a day old chick that died from a natural infection of *Salmonella* Gallinarum. 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 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 and by the dilution factor (CFU = No. of colony × Volume × Reciprocal of Dilution factor) (Miles and Misra, 1938). Challenge of the quails was done orally using sterile syringes. Group A quails received a dose of 1×10^6 organisms/0.2 ml of nutrient broth, group B quails received a dose of 1×10^4 organisms/0.2 ml of nutrient broth, while group C quails received a dose of 1×10^2 organisms/0.2 ml of nutrient broth. Group D 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 (1938).

2.6. Pathological Examinations

Mortality was recorded. A detailed post-mortem examination was performed on dead birds, and on birds surviving to the end of the experiment, following euthanasia by cervical dislocation. Gross lesions were observed, recorded and photographed. Samples of the liver, spleen, heart, small and large intestines, lung, ovary, pancreas and caecal tonsil showing gross lesions were collected and fixed in 10% buffered neutral formalin for at least 48 hours. Following fixation, the tissues were trimmed, placed in cassettes and dehydrated in graded concentrations of alcohol (70%, 80%, 90% and 100%) using an automatic tissue processor (STP 120). The tissues were cleared with xylene, embedded in molten paraffin wax block and labelled appropriately (Oladele *et al.*, 2008). The tissue paraffin blocks were sectioned at 5 µm thick, using a microtome. The sectioned tissues were mounted on a grease-free, clean glass slide, dried at room temperature and stained with haematoxylin and eosin (H & E) stains. The slides were evaluated using light microscope at ×40 and ×100 magnifications in order to determine any pathological changes. Photomicrographs of lesions from the different organs were taken, transferred to a computer and labelled

appropriately (Hossain *et al.*, 2006).

3. Results and Discussion

Mortality was observed in all infected groups (A, B and C), with mortality rates of 35%, 30% and 30%, respectively (Table 1). No mortality was recorded in group D (control).

Table 1. Mortality pattern of Japanese quails after infection with *Salmonella enterica* serovar *Gallinarum*.

Day after infection	Mortality rate per group			
	Group A (10 ⁶)	Group B (10 ⁴)	Group C (10 ²)	Group D (control)
5	3	-	-	-
7	4	3	3	-
8	3	3	3	-
9	2	1	2	-
10	1	2	1	-
11	1	1	1	-
15	-	1	-	-
18	-	1	1	-
20	-	-	1	-
Total	14 (35%)	12(30%)	12(30%)	-

Table 2. Gross lesions in different groups of quails experimentally infected with *Salmonella enterica* serovar *Gallinarum*.

Number and percentage of birds with lesions in each infected group				
Organ/Lesion	Group			
	A (10 ⁶)	B (10 ⁴)	C (10 ²)	D (control)
<i>Liver</i>				
Hepatomegaly	5(12.5%)	5(12.5%)	4(10%)	0
Congestion	19(47.5%)	26 (65%)	17(42.5%)	0
Bronze discoloration	2(5%)	1(2.5%)	1(2.5%)	0
Friable	-	-	2(5%)	0
<i>Lung</i>				
Congestion	19(47.5%)	26(65%)	17(42.5%)	0
Edema	3(7.5%)	6(15%)	5(12.5%)	0
<i>Spleen</i>				
Splenomegaly	6(15%)	4(10%)	3(7.5%)	0
<i>Kidney</i>				
Swelling	-	1(2.5%)	-	0
<i>Ovarian Follicle</i>				
Egg yolk peritonitis	1(2.5%)	1(2.5%)	-	0
Congestion	1(2.5%)	2(5%)	1(2.5%)	0
<i>Peritoneum</i>				
Hydroperitoneum (ascites)	1(2.5%)	-	-	0



Plate I. Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10⁴ of *Salmonella enterica* serovar *Gallinarum*. Note hepatomegaly and bronze liver (*)

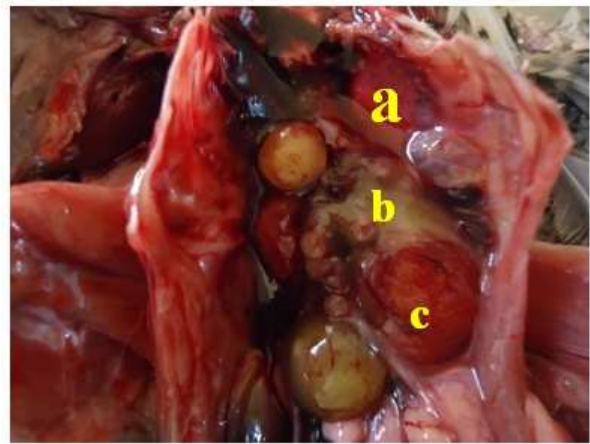


Plate II. Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10⁶ of *Salmonella enterica* serovar *Gallinarum*. Note congested lung (a), egg yolk peritonitis (b) and congested ovarian follicle (c).

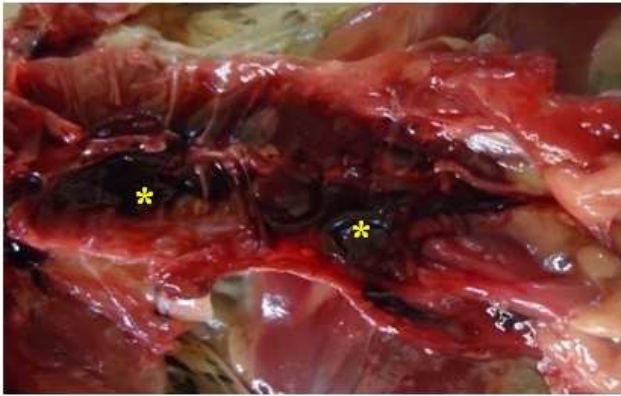


Plate III. Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica serovar Gallinarum*. Note enlarged and congested kidneys (*).

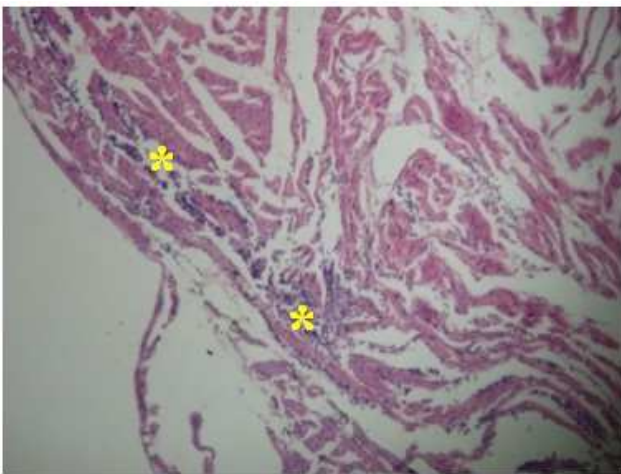


Plate IV. Photomicrograph of the heart of Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica serovar Gallinarum*. Note: Generalized cellular infiltration.

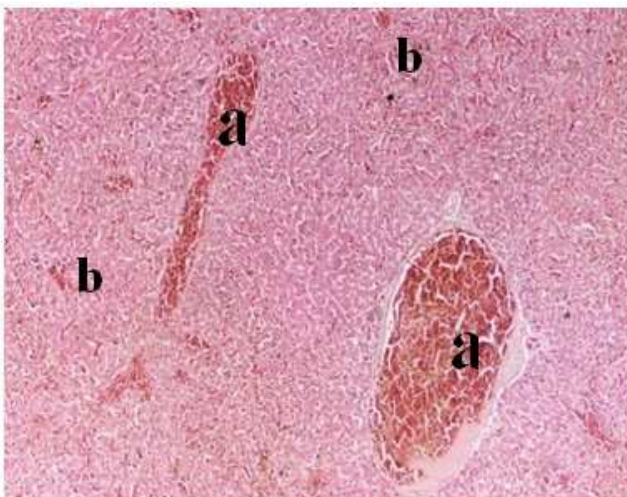


Plate V. Photomicrograph of the liver of Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica serovar Gallinarum*. Note severe congestion (a) and haemorrhages (b). H & E. $\times 40$.

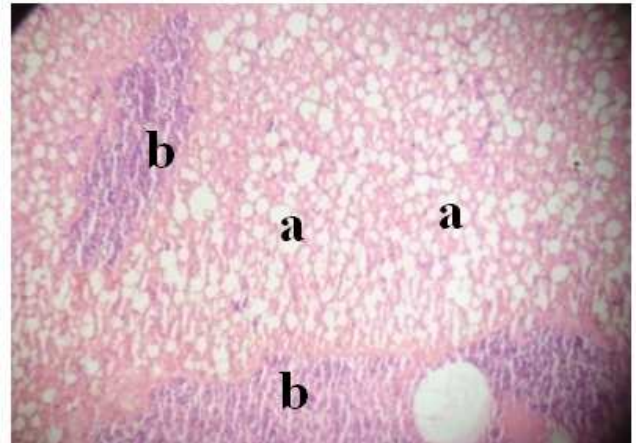


Plate VI. Photomicrograph of the liver of Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica serovar Gallinarum*. Note generalized vacuolation due to fatty infiltration (a) and cellular infiltration (b). H & E. $\times 100$.

A mortality rate of 30-35% was recorded in this study, which is a little lower but still significant as that in chickens (10- 93%) reported by Shivaprasad (1996). The mortality in this study was mostly that of sudden death, suggesting that the infection might have been peracute to acute. The mortality started five days post-infection and persisted till day twenty. The high mortality recorded in this experiment indicated that Japanese quails are also susceptible to *Salmonella enterica serovar Gallinarum* similar to the chickens. The gross lesions and histopathological findings observed in Japanese quails (*Coturnix coturnix japonica*) in this study were consistent with previous studies in chickens (Garcia *et al.*, 2010). The gross lesions of hepatomegaly, splenomegaly, swollen kidneys, congested liver, lung and ovarian follicle, bronze liver, egg yolk peritonitis, edematous lung and hydroperitoneum (ascites), observed in this study were also similar to those in chickens reported by previous researchers (Beyaz *et al.*, 2010; Garcia *et al.*, 2010). The gross lesions are highly indicative of septicemic infection. The histopathological findings (cellular infiltration of the liver and heart, vacuolation of hepatocytes and congested liver and lung) in this study were also similar to previous works in chickens (Hossain *et al.*, 2006; Nwiyi and Omadamiro, 2012). This is the first time these findings were reported in quails in Zaria, Kaduna State, Nigeria to the best of our knowledge. The organism was isolated from the liver, spleen, heart and intestine of the quails at the end of the experiment; implying a septicemic condition.

4. Conclusion

This study demonstrates that *Salmonella Gallinarum* caused a septicemic disease with distribution of the organism in major organs and characterized by high mortality, congestion and enlargement of various organs in Japanese quails (*Coturnix coturnix japonica*). Other pathological findings, such as bronze liver, hepatomegaly, splenomegaly and congested liver, lung and ovarian follicle in this study

indicated that *Salmonella Gallinarum* produces a disease similar to that in chickens.

Recommendations

Vaccinating Japanese quails reared in the country is highly recommended as a preventive measure against fowl typhoid. Farmers keeping quails should adhere to strict biosecurity measures as means of prevention and control of fowl typhoid in quail farm.

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