



Clinicopathological Features in Japanese Quails (*Coturnix coturnix japonica*) Inoculated with *Pasteurella multocida* Serotypes A: 1, 3 and 4

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ABSTRACT

This experimental study was conducted to document the clinicopathologic features observed in Japanese quails inoculated with *P. multocida* serotypes A: 1, 3 and 4. Thirty 6-week-old Japanese quails (male and female) were divided into five groups (A, B, C, D and E) of 6 quails each. Groups A, B, C and D were further sub-divided into three sub-groups, with each group consisting of two quails. Quails in groups A, B, C and D were inoculated with 0.1ml of *Pasteurella multocida* serotypes A: 1, 3 and 4 at concentrations 10⁶, 10⁷ and 10⁸ CFU/quail respectively through intramuscular route in the pectoral muscle. Group D was inoculated with fowl cholera vaccine strain A: 1 of *P. multocida*, while quails in group E, the uninfected control were inoculated with normal saline. All the infected Japanese quails manifested clinical signs such as weakness, inappetence and sudden death. Hundred percent mortality was observed at concentrations of 10⁸, 10⁷, in groups A and B. No mortality was noticed in group D (vaccine strain) and group E (uninfected control). Gross lesions observed were petechial and ecchymotic haemorrhages on the heart and breast muscles, congested heart, liver and lungs in affected quails. Histopathological lesions observed in group A, B and C were generalized mononuclear cellular infiltration and pulmonary congestion in the lungs. It was observed that Japanese quails were susceptible to fowl cholera caused by serotypes A: 1, 3 and 4 and need to be vaccinated with polyvalent fowl cholera vaccine containing serotypes A: 1, 3 and 4.

Key words: Clinicopathologic, *Pasteurella Multocida*, Quail, Serotypes.

INTRODUCTION

Japanese quails are highly prolific, they have short generation time and the meat has low cholesterol (Haruna et al., 1997). Japanese quails have been reported to be generally resistant to common diseases that may adversely affect other birds; but they can still be affected by several common poultry diseases (National Research Council, 1991). One of the major problems faced by quail farmers was the susceptibility of Japanese quails to infectious, non-infectious and parasitic diseases. There were documented reports that Japanese quails were susceptible infectious diseases such as fowl cholera, colibacillosis salmonellosis, Newcastle disease and ulcerative enteritis (Naveen and Arun, 1992; Lima et al., 2004; Oladele et al., 2008). Odugbo et al. (2004) reported a massive mortality in Japanese quail farm in Vom, Nigeria due to *P. multocida* serotype A: 4 in 2004. During the outbreak, up to 80% mortality was recorded as a result of fowl cholera disease on the farm. Mwankwo et al. (2008) observed that a recurrent outbreak of fowl cholera in a Japanese quail farm in Vom, Nigeria was attributed to rats (*Rattus rattus*) co-habiting quail houses. There was extensive literature on the incidence, prevalence clinical manifestations of fowl cholera in other types of birds such as chickens, turkeys, ducks and geese, but there was paucity of information on the disease and the role of serotypes of the causative agent (*P. multocida*) in Japanese quails in Nigeria. This study therefore seeks to document the clinicopathologic features observed in Japanese quails inoculated with *P. multocida* serotypes A: 1, 3 and 4.

MATERIAL AND METHODS

Management and housing of quails

Thirty 6-week-old Japanese quails (*Coturnix coturnix japonica*) (male and female) were supplied by the National Veterinary Research Institute, Vom, Nigeria. The Japanese quails were divided into groups A, B, C, D and E of 6 quails

ORIGINAL ARTICLE
 pii: S232245681500002-4
 Received: 25 Jan 2015
 Accepted: 28 Feb 2015

each. Groups A, B, C and D were further sub-divided into three sub-groups, with each group consisting of two Japanese quails. Quails in each group were housed separately in wire mesh cages with floor space of 35 cm by 35 cm measurement in size in an enclosed house which was cleaned and disinfected weekly. The birds were fed on layer mash and water given ad libitum.

Source of *Pasteurella multocida*

The *P. multocida* serotypes A: 1, 3 and 4 were recovered by the authors from chickens with clinical cases of fowl cholera in Jos, Nigeria in 2012. The isolates were confirmed by biochemical test, Microbact, PCR and Multiplex PCR. They were serotyped at United State Department of Agriculture in USA.

Inoculation of Japanese quails

All groups (A, B, C, D and E) were used for the pathogenicity study. The *P. multocida* serotypes were reconstituted into three different concentrations for groups A, B and C, then, every quail in first sub-group of group A administered 0.1 ml intramuscularly of 10^6 CFU (serotype A: 1 - field strain) contained in Triptose Soya Broth, the second sub-group received 10^7 CFU of the same serotype and the last sub-group received 10^8 CFU of serotype A: 1, the administration of serotypes A: 3 and A: 4 of *P. multocida* at the same doses were replicated for sub-groups of group B and C respectively. The birds in group D were injected with *P. multocida* serotype A: 1 (fowl cholera vaccine strain) at the same concentrations of 10^6 , 10^7 , and 10^8 . Birds in group E were injected with normal saline 0.1 ml intramuscularly.

Clinical and pathological examinations

Japanese quails in all the groups were observed for clinical signs and mortality post inoculation. Postmortem examinations were conducted on quails that died and tissue section of the spleen, liver, heart and lungs were prepared for histology. The section of grossly affected organs were fixed in 10% buffered formalin; paraffin embedded tissues were sectioned at 5 microns, stained with haematoxylin and eosin (H & E) and mounted on glass slides and examined under light microscope at x200 and x400 respectively.

Statistical Analysis

The entry and sorting of primary data was performed with Microsoft excel, 2010. Descriptive statistical analysis was conducted using Statistical Package for Social Sciences SPSS (version 12.01) (2004) and the results were summarized as percentages in tables.

RESULTS

All Japanese quails in groups A, B, and C became sick within 24 h. Weakness, inappetence, somnolence and sudden death were also observed in the three groups (Table 1). No clinical signs and mortality were recorded in groups D (vaccine strain, that is, *P. multocida* serotype A: 1) and E (uninfected control). Congested heart, liver and lungs were noticed in all the 3 groups (A, B and C). Petechial and ecchymotic hemorrhages were noticed on the heart and breast muscles of birds in groups A and C (Table 2, Figures 1 and 2). Mortality rates were 100% for concentrations of 10^8 and 10^7 CFU in group A and B, while 0% mortality recorded for 10^6 CFU groups B and C. No mortality was noticed in group D (Vom vaccine strain, that is, *P. multocida* strain) and group E (uninfected control) (Table 3). Histopathological findings indicated a moderate to severe lymphocytic, heterophilic and macrophages cellular infiltration in the cardiac muscles of experimentally infected Japanese quails (Figure 3).

DISCUSSION

The current experimental study revealed that Japanese quails (*Coturnix coturnix japonica*) were susceptible to *P. multocida* serotypes A: 1, 3 and 4. Different clinical manifestations and pathological lesions were observed in all the quails during the 3-week study period. Clinical signs such as weakness, in appetite, ruffled feathers and somnolence observed in quails in groups A, B and C were indications of acute infections which are characteristic features of acute form of fowl cholera. This finding concurred to the report of Akpavi et al (2011) who noticed the manifestation of these signs within 24 h post inoculation of *P. multocida* serotype A: 4. In a similar study conducted in India by Kumar et al. (2004), they found majority of *P. multocida* belonging to serotypes A: 1, A: 3 and A: 4 were associated with fowl cholera. Previous report by Panigrahy and Glass (1982) indicated that *P. multocida* serotype A: 3 cause acute fowl cholera with high mortality in natural infections in three flocks of quails in America, one involving pharaoh quails (*Coturnix coturnix*) and two involving bobwhite quails (*Coturnix virginianus*).

Table 1. Clinical signs of 6-week-old Japanese quails Inoculated with *Pasteurella multocida* Serotypes A: 1, A: 3 and A:4.

Clinical signs	Group A	Group B	Group C	Group D	Group E
Weakness	6 (100%)	3 (50%)	6 (100%)	0 (0%)	0 (0%)
Inappetence	6 (100%)	6 (100%)	3 (50%)	0 (0%)	0 (0%)
Somnolence	6 (100%)	3 (50%)	6 (100%)	0 (0%)	0 (0%)

Table 2. Gross lesions of 6-week-old Japanese quails Inoculated with *Pasteurella multocida* Serotypes A: 1, A: 3 and A:4.

Gross lesions	Group A	Group B	Group C	Group D	Group E
Congested heart	6 (100%)	6 (100%)	3 (50%)	0 (0%)	0 (0%)
Congested liver	6 (100%)	6 (100%)	6 (100%)	0 (0%)	0 (0%)
Congested lungs	3 (50%)	3 (50%)	3 (50%)	0 (0%)	0 (0%)
Petechial & ecchymotic hemorrhages of heart	6 (100%)	6 (100%)	0 (0%)	0 (0%)	0 (0%)
Hemorrhages of breast muscles	3 (50%)	3 (50%)	0 (0%)	0 (0%)	0 (0%)

Table 3. Mortality of Japanese quails after inoculated with *Pasteurella multocida* serotypes A: 1, A: 3 and A: 4 on day 7.

Groups	Mortality
A	2 at 10^8 CFU
	2 ,, 10^7 CFU
	1 ,, 10^6 CFU
B	2 ,, 10^8 CFU
	2 ,, 10^7 CFU
	0 ,, 10^6 CFU
C	2 ,, 10^8 CFU
	0 ,, 10^7 CFU
	0 ,, 10^6 CFU
D	0 ,, 10^8 CFU
	0 ,, 10^7 CFU
	0 ,, 10^6 CFU
E	0 ,, 10^8 CFU
	0 ,, 10^7 CFU
	0 ,, 10^6 CFU

The widespread pathological lesions observed in visceral organs of the quails could possibly explain the profound debilitation observed in birds suffering from acute form of fowl cholera. Similar findings were previously reported by Odugbo et al. (2004); Shilpa and Verma (2006). Akpavi et al. (2011) reported 92% mortality in Japanese quails inoculated with *P. multocida* serotype A: 4; their figure was rather high compared to the 33.3% recorded group C which was inoculated *P. multocida* serotype A: 4 in this study. The difference could be attributed to the concentrations and pathogenicity of *P. multocida* inoculum which led to high susceptibility of the experimental quails in the present study. Myint and Carter (1988) also reported 60% mortality in a natural outbreak of fowl cholera in quails in Burma. Bermudez et al. (1997) reported 99% mortality in Bobwhite quail (*Colinus virginianus*). In this study, mortality in the Japanese quails was first noticed only on day seven post inoculation. This report is at variance with the findings Akpavi et al. (2011) who reported that mortality was first noticed one day post inoculation and lasted five days with highest mortality rate on day one post inoculation. High mortality was recorded in groups of quails inoculated with *P. multocida* serotypes A: 1, 3 and 4 at concentration 10^8 on day five, this finding showed that there is a correlation between the severity of fowl cholera and high concentration of *P. multocida* inoculum. This report differs from the findings Akpavi et al. (2011) who reported that there was no correlation between the dose of inoculum and mortality rate.

Histopathological findings in the present study indicated a moderate to severe cellular infiltration in the heart, lungs liver and spleen. This could possibly signify that the birds were responding to the inciting bacterial agent which is a common characteristic in the acute form of fowl cholera. Shilpa and Verma (2006) and Akpavi et al. (2011) observed a

similar histopathological changes such as cellular and heterophilic infiltration in the lungs, liver, spleen and heart. Glisson et al. (1989) had earlier reported multifocal splenic and hepatic necrosis and interstitial pneumonia in natural outbreak of fowl cholera involving three flocks of Japanese quails.

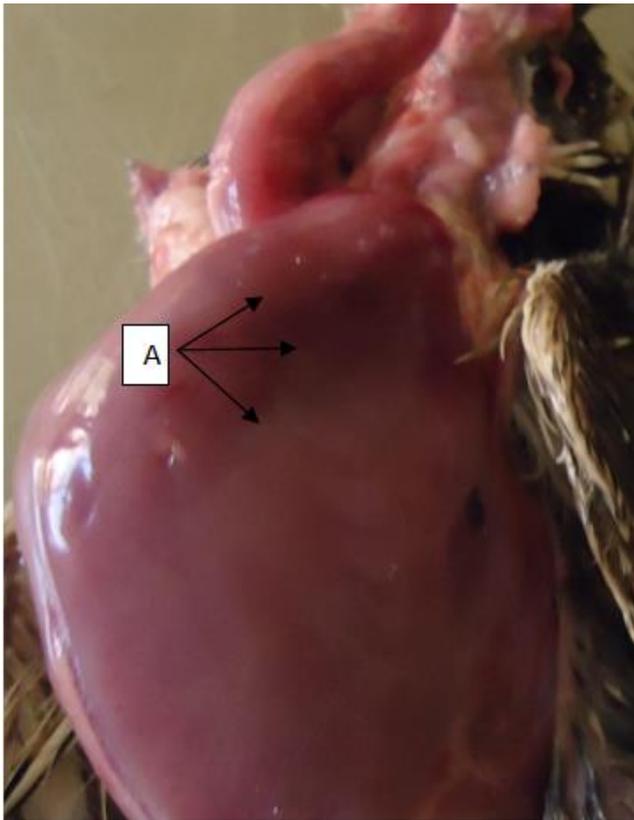


Figure 1. Photograph of a 6-week old Japanese quail inoculated intramuscularly with *P. multocida* A: 3 at 10^8 CFU, Congestion of breast muscles of Japanese quail

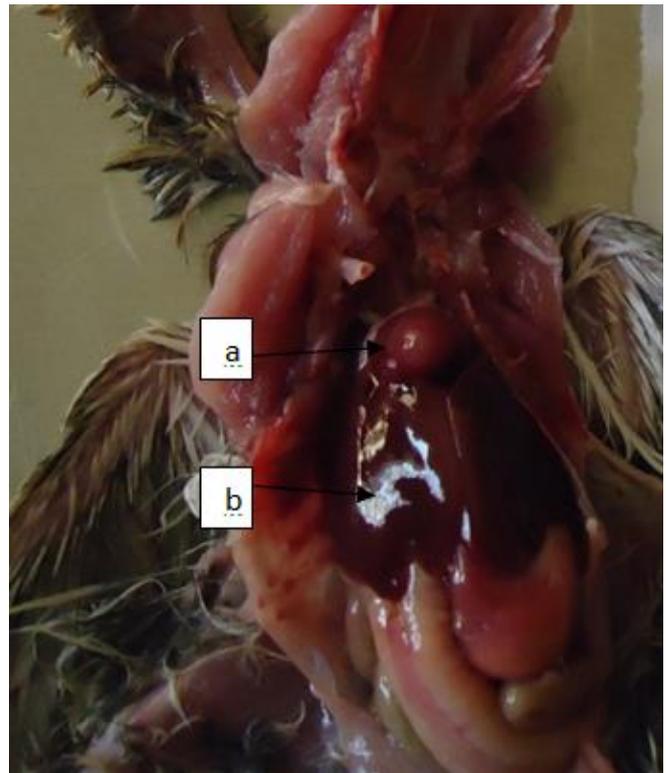


Figure 2. Photograph of a 6-week old Japanese quail inoculated with *Pasteurella multocida* serotype A: 4 at 10^8 CFU, a: congested heart, b: congested liver

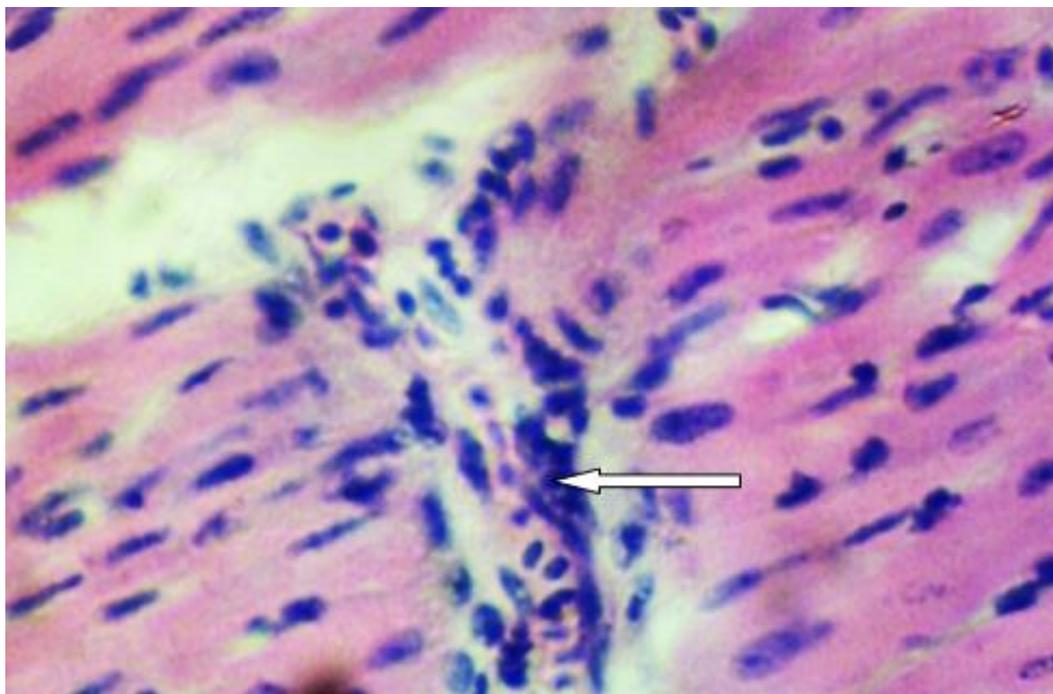


Figure 3. Histopathologic view of cardiac muscle of a 6-week old Japanese quail inoculated with *P. multocida* A: 4 at 10^8 concentration, arrow showing infiltrated inflammatory cells in an area of myocardial necrosis. H and E Stain. x400

CONCLUSION

In conclusion, from this study, it has been revealed that Japanese quails are highly susceptible to infections with *P. multocida* serotypes A: 1, 3 and 4 and can cause per acute and acute disease with high mortality. It is therefore recommended that Japanese quails should be vaccinated against fowl cholera with a polyvalent vaccine consisting of *P. multocida* serotypes A: 1, 3 and 4 in order to protect them against *P. multocida* infections.

ACKNOWLEDGEMENTS

The authors acknowledge the valuable contribution of staff of the departments of Central diagnostic and Molecular biology, National Veterinary Research Institute, Vom, Nigeria, for carrying out post mortem on the carcasses and characterization of the different serotypes of *P. multocida* respectively.

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