AN OUTBREAK OF AVIAN PASTEURELLOSIS IN A QUAIL FARM IN TAMIL NADU

S.U. Abdul Latheef*[1], S. Ramesh [2], S. Hemalatha [3], A. Ashok Kumar [4], Ganne Venkata Sudhakar Rao [5] and G. Balakrishnan[6]

Department of Veterinary Pathology

Madras Veterinary College

Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600 007

ABSTRACT

An outbreak of pasteurellosis was recorded in a local Japanese quail farm in Tamil Nadu with a flock capacity of 20,000 birds per batch. Mortality was reported in new arrival of 2000 birds of 14 week old quails. Mortality was recorded to be increasing from third day to 10th day of arrival with a total mortality of 410 birds at the rate of 20 birds per day for three consecutive days and 30 to 60 for seven consecutive days with the history of depression, ruffled feathers, discharge of mucous from the mouth and nostrils, anorexia, diarrhoea and respiratory distress. Necropsy was performed on dead birds which revealed numerous greyish necrotic foci scattered on the entire parenchyma of the liver on gross examination. Heart showed petechial haemorrhages on the pericardium while other organs namely kidney, intestine, spleen and lungs showed only congestion. Impression smear of the liver revealed presence of bipolar organisms on microscopic examination. Cultural examination of heart blood swab and PCR assay carried out on liver tissues confirmed the presence of *Pasteurella multocida*. Based on the postmortem examination and laboratory findings, diagnosis was confirmed as pasteurellosis and all the other ailing birds were treated with enrofloxacin orally @ 5 mg/kg body weight for 7 days. The treated birds became active with normal feeding habits after 10 days of treatment.

Key words: Japanese quails- gross pathology- culture test-PCR assay- enrofloxacin

Received : 28.11.2022 Revised : 23.02.2023 Accepted : 28.02.2023

INTRODUCTION

Japanese quails have a short generation period, a rapid rate of reproduction, and low-cholesterol meat (Haruna *et al*., 1997). According to reports, Japanese quails were typically immune to common diseases that could affect other birds, although they are still susceptible to a variety of common poultry ailments (National Research Council, 1991). The sensitivity of Japanese quails to infectious, non-infectious and parasitic diseases was one of the main issues faced by quail farmers.

[1]M.V.Sc Student, Corresponding author Email id: drlatifivpm@gmail.com

[2,3]Professor

[4]Professor, Dept. of Poultry Science, MVC, Chennai

[5]Professor and Head

[6]Professor and Head, Central University Laboratory, Madhavaram Milk Colony, Chennai – 600 051

Japanese quails were reportedly prone to infectious diseases like fowl cholera, colibacillosis, salmonellosis, Newcastle disease, and ulcerative enteritis. (Oladele *et al*., 2008). Fowl cholera was an infectious bacterial disease caused by *Pasteurella multocida* that affects both domesticated and wild bird species and impairs the profitable production of chicken (Raji *et al*., 2010). Although it typically emerges as a septicemic disease with significant morbidity and mortality, it could also occur as chronic or benign disorders (Glisson *et al*., 2008). Turkeys are typically more susceptible among domesticated birds, followed by chicken and ducks (Shilpa *et al*., 2015). In most cases, fowl cholera affects birds between the ages of 10 and 13 weeks, with birds younger than 2 weeks being a rare exception (Glisson *et al*., 2008). Quails and young birds were resistant to *Pasteurella multocida* infection, according to NRC (1991). Acute fowl cholera's obvious clinical symptoms, which include depression, ruffled feathers, fever, anorexia, mucous discharge from the mouth, diarrhoea and respiratory distress but will not manifest until a very late stage in the infection (Rhoades and Rimler, 1990). In chickens, mortality from comb and wattle cyanosis can range from 0 to 20 %. (Glisson *et al*., 2008). Grossly, the infected dead birds had severe congestion of the visceral organs with necrotic hepatitis, congestion and haemorrhages in the intestinal mucosa and petechial haemorrhages on the pericardium with serofibrinous pericarditis (Glisson *et al*., 2008).

According to Babu Prasath *et al*. (2018), a Japanese quail farm in Thanjavur, Tamil Nadu, experienced a significant amount of mortality in 2018. Up to 30 % of deaths on the farm during the outbreak were attributed to the fowl cholera disease. There was only limited information available on the occurrence and clinical signs of fowl cholera in Japanese quails, compared to other types of birds such chickens, turkeys, ducks, and geese (Glisson *et al*., 2008). This study documents the occurrence of acute fowl cholera in 14 weeks old Japanese quail chicks.

MATERIALS AND METHODS

A Japanese quail farm at Ambur in Vellore district of Tamil Nadu reported 410 deaths of Japanese quails with the history of sudden death in a span of 10 days. The newly arrived 12 weeks old Japanese quails were kept in grower cage close to the grower flocks which suffered fowl cholera recently. Birds were not vaccinated against any viral infections and not treated with drugs. A few dead birds were brought to the Department of Veterinary Pathology, Madras Veterinary College, Chennai-7, Tamil Nadu, India for necropsy diagnosis. Detailed necropsies with systemic examination were carried out on the carcasses. Swabs from heart and lungs were collected for bacteriological examination and inoculated in nutrient broth and on blood agar. Bacterial colonies were Gram stained and identified as per standard methods (Quinn *et al*., 2011). Impression smears from lungs and heart blood were collected for cytological study. Representative tissue samples of lungs, heart, spleen and liver were collected from all the

quails in 10 % formalin for histopathological studies and without preservative for carrying out PCR assay. PCR assay was carried out for KMT1 gene with KMT1T7 (5'-ATC-CGC-TAT-TTA-CCCAGT-GG-3') and KMT1SP6 (5'-GCT-GTA-AAC-GAA-CTCGCC-AC-3') primers (Townsend *et al*., 1998).

Touch impression smears were stained with Grams and Leishman's staining technique. Tissue samples collected during necropsy were fixed in 10 % buffered formalin and 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 (Bancroft and Gamble, 2008).

RESULTS

Mortality was reported to be increasing from third day to 10th day with a total mortality of 410 birds, at the rate of 15 birds per day for three consecutive days and 30 to 60 for 7 consecutive days with the history of depression, ruffled feathers, discharge of mucous from the mouth and nostrils, anorexia, diarrhoea and respiratory distress (Fig.1). No clinical signs could be seen in some birds.

On external examination, quails appeared thin with pale mucous membrane. Necropsy was performed on dead birds which revealed on gross examination (Fig. 2a-2d), numerous greyish necrotic foci scatterred on the entire parenchyma of the liver. Heart showed petechial haemorrhages on the pericardium. Some birds revealed petechial and ecchymotic haemorrhages on the breast muscles. Other visceral organs namely kidney, intestine, spleen, liver and lungs showed only congestion. In addition, liver appeared mottled appearance. Lungs appeared dark brownish with firm consistency. However some dead birds did not show any gross lesions.

Fig 1. Mortality observed in Ambur quail farm

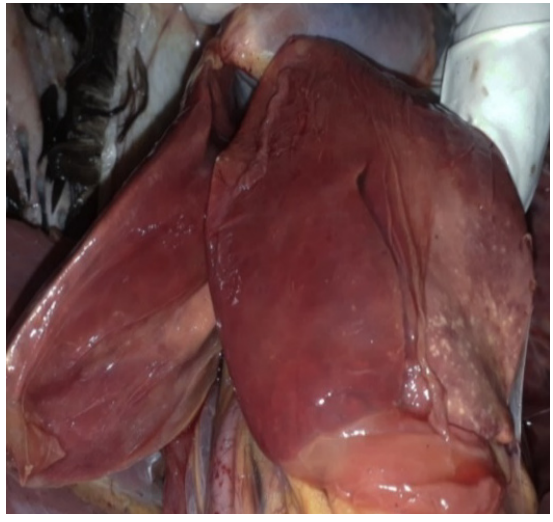

Fig 2a. Enlarged and multiple necrotic foci in Liver

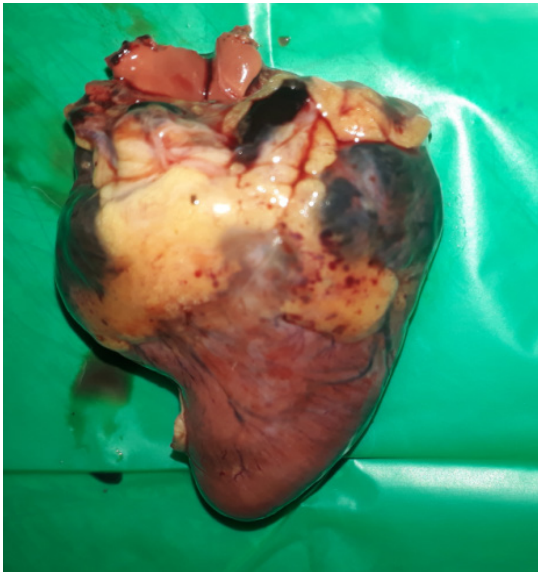

Fig 2b. Subepicardial haemorrhages in heart

Fig 2c. Hyperemia of duodenum

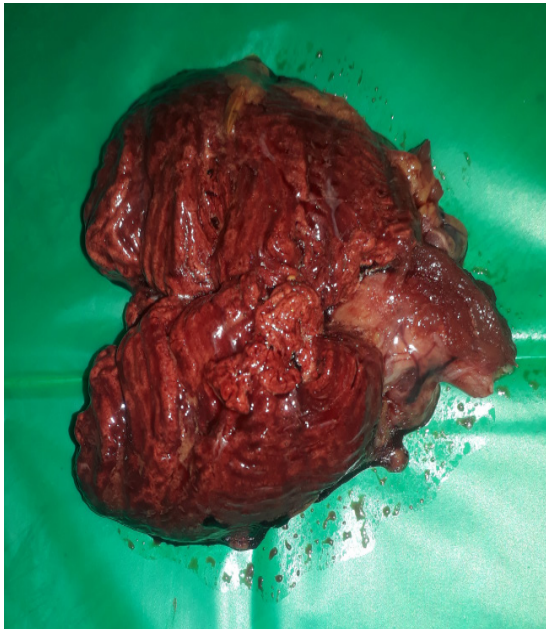

Fig 2d. Lung with extensive haemorrhage

The impression smear of the heart blood revealed presence of numerous, small, typically bipolar organisms on microscopic examination (Fig. 3).

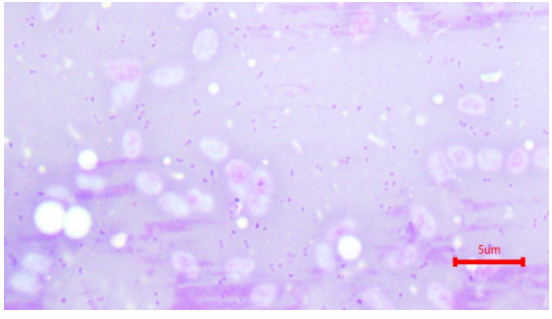

Fig 3. Heart blood smear stained with Leishman – Giemsa indicates the presence of bipolar organisms

Histopathological studies revealed diffuse mild congestion in kidney, intestine, spleen, liver, lung and duodenum. Heart showed moderate to severe lymphocytic, heterophilic and macrophages cellular infiltration in the cardiac muscles. Liver showed diffuse vacuolar changes of hepatocytes and multifocal periportal coagulative necrosis with subacute hepatitis (Fig.4). Liver revealed lymphoid cell infiltration in parenchyma (Fig. 5).

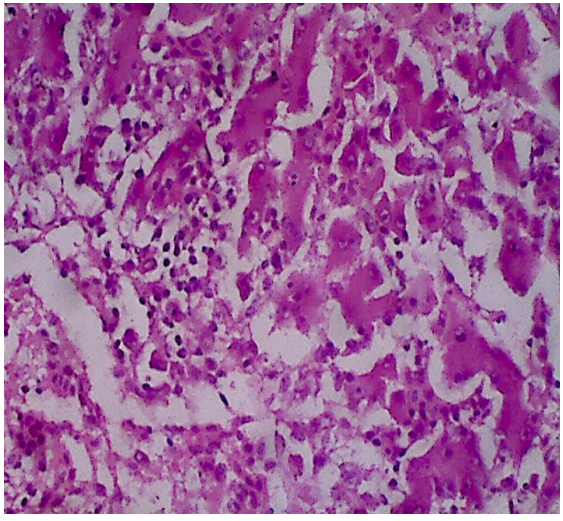

Fig 4. Liver - Multifocal periportal coagulative necrosis with subacute hepatitis

The organisms from swabs of heart produced turbidity on nutrient broth. They were found to grow on nutrient broth and in blood agar. In blood agar, it produced whitish, opaque, round, flat, translucent colonies (Fig. 6). The isolates consistently produced acid from fermenting dextrose, sucrose and mannitol but not maltose or lactose. The organism was found to be non-motile. It was found to be indole, oxidase and catalase positive and urease negative.

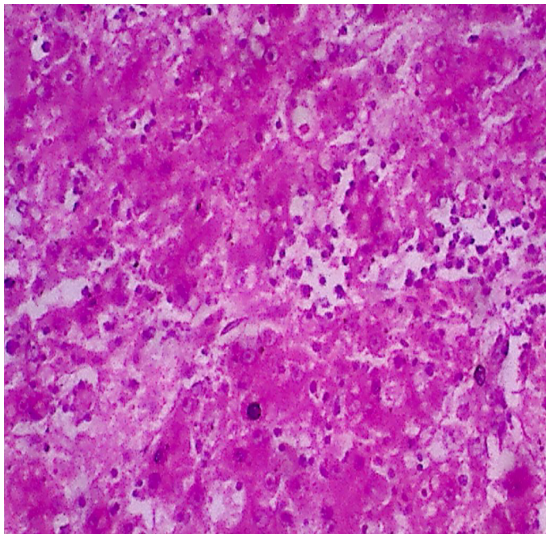

Fig 5. Liver- Lymphoid cell infiltration in parenchyma

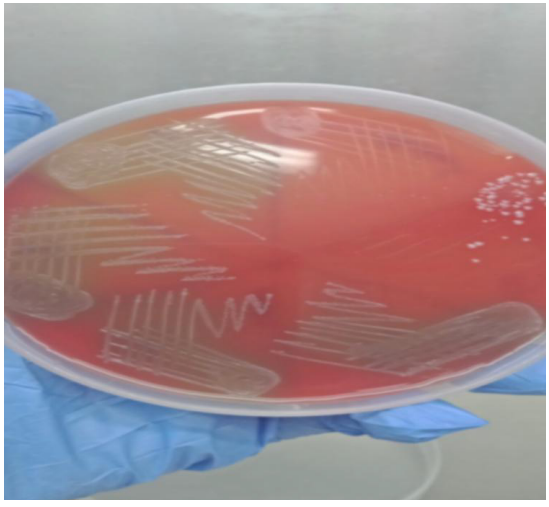

Fig 6. Presence of pinpoint dew drop colonies in blood agar plate

Polymerase chain reaction (PCR) revealed amplification of KMT1 gene with 460 bp in all isolates which is consistent with *Pasteurella multocida* (Fig. 7).

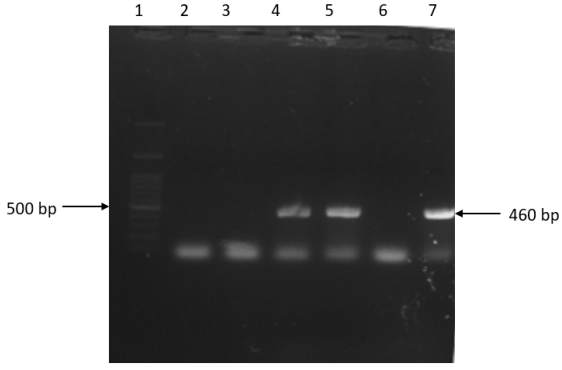

Fig 7. Agarose gel electrophoresis Amplification of 460 bp PCR product for capsular antigen of *Pasteurella multocida.*
Lane 1 – 100 bp DNA Ladder, Lane 2 and 3 Negative samples, Lane 4 and 5 Positive samples, Lane 6 - Negative control, Lane 7- Positive control

Cultural examination of heart blood swab and PCR assay carried out on liver tissues confirmed the presence of *Pasteurella multocida*. Based on the postmortem examination and laboratory findings, diagnosis was confirmed as pasteurellosis and all the other ailing birds were treated with enrofloxacin orally @ 5 mg/kg b wt for 7 days. The treated birds became active with normal feeding habits after 10 days of treatment.

DISCUSSION

Fowl cholera in quails was first reported by Hinshaw and Emlen (1943). According to NRC (1991), chicken under 16 weeks old and quails were claimed to be resistant to fowl cholera, but in the present case, fowl cholera affected 14 week old quails. In natural quail cholera outbreaks, mortality can range from 60 % (Miguel *et al*., 1998) to 99 % (Bermudez *et al*., 1997). In this study, mortality in the Japanese quails was first noticed in 14 weeks old bird's grower cages. High mortality of about 20 % at 8 days recorded in this study was in concordance with the earlier research reports (Miguel *et al*., 1998; Babu Prasath *et al*., 2018). Some quails which died without any clinical signs in the present study indicated the acute infection and were similar with the findings of Odugbo *et al*. (2004) in four weeks old Japanese quails died of acute pasteurellosis. The clinical signs observed in this study were similar with findings of Kumar *et al*. (2004), Akpavi *et al*. (2011) and Yakubu *et al*. (2015).

The gross lesions observed in this study were similar with the earlier findings (Goto *et al*., 2001; Oladele *et al*., 2008; Akpavi *et al*., 2011; Babu Prasath *et al*. (2018), in Japanese quails. Petechial and ecchymotic haemorrhages on pericardium and breast muscles observed in this study were similar with earlier findings (Yakubu *et al*., 2015) in Japanese quails.

Histopathologically, liver with multifocal coagulative necrosis and heterophilic infiltrations pathognomonic to avian pastuerellosis observed in this study coincided with the findings of others (Glisson *et al*., 2008; Yakubu *et al*., 2015). The present

study indicated a cellular infiltration in the heart and liver. 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 change such as cellular and heterophilic infiltration in the lungs, liver, spleen and heart.

In this study, the gross lesions were not marked in some Japanese quails affected with acute fowl cholera when compared to other poultry species. Pneumonic lesions recorded in this study were very similar with the reports of Glisson *et al*. (2008) and Shilpa *et al*. (2015). Though the pneumonic lesions were more prominent in turkeys than in other species in acute pasteurellosis (Glisson *et al*., 2008), the present study described the subacute bronchopneumonia in acute pastuerellosis infection in 14 weeks old Japanese quails which was in accordance with the earlier report of Babu Prasath *et al*. (2018), in 8 day old quail chicks.

The PCR assay with 460 bp product was in consistent with previous findings of Townsend *et al*. (1998). *Pasteurella multocida* is a heterogeneous species and pathogenicity of individual strains were highly variable and the susceptibility to *Pasteurella multocida* varies considerably among avian species as recorded by Christensen and Bisgaard (2000). The results indicated that high mortality is due to *Pasturella multocida* infection. The predisposing factors need to be analysed further, as *Pasteurella multocida* initiates its pathogenesis with certain conducive factors. In this present study, few of the following predisposing factors might be involved in the cause of outbreak. Firstly, the quails which were affected with fowl cholera were kept in place near the previous outbreak of fowl cholera. Secondly, improper disinfection of utensils which were used previously in outbreak flocks. Thirdly, stress due to transportation and loading makes the quails more vulnerable for the establishment of *Pasteurella multocida* infection.

Based on the postmortem examination and laboratory findings, diagnosis was confirmed as pasteurellosis and all the other ailing birds were treated with enrofloxacin orally @ 5 mg/kg b wt for 7 days. The treated birds became active with normal feeding habits after 10 days of treatment.

This present study concludes that the infection with *Pasteurella multocida* can occur in 14 weeks old quails of grower birds/cages when remarkable predisposing factors coexist. It also records that *Pasteurella multocida* infection in Japanese quails leads to severe bronchopneumonia on par with turkeys.

REFERENCES

Akpavi, V., Abdu, P.A., Mamman, P.H. and Sa'idu, L. (2011). Clinicopathological features in Japanese quails (*Coturnix coturnix japonica)* infected with *Pasteurella multocida*. *Sahel Journal of Veterinary Science*, **19**:15-20.

Babu Prasath, N., Selveraj, J., Ponnusamy, P. and Sasikala, M. (2018). An outbreak of pasteurellosis in Japanese quail chicks (*Coturnix coturnix japonica*). *Indian Journal of Animal Health*, **57**(2): 189-194.

Bancroft, J.D. and Gamble, M. (2008). Theory and Practice of Histological Techniques. 6th Edn., Churchill Livingstone, Philadelphia.

Bermudez, A.J., Munger, L.L. and Ley, D. H. (1997). *Pasteurellosis* in Bobwhite quails. *Avian Diseases*, **35**: 618-620.

Christensen, J.P. and Bisgaard, M. (2000). Fowl cholera. *Revue Scientifique Et Technique De Le. Bulletin - Office International Des Epizooties*, **19**: 626 – 637.

Glisson, J.R., Hofacre, C.L. and Christensen, J. P. (2008). Fowl cholera. In Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L. R. McDougald and D. E. Swayne Eds. Diseases of Poultry, 12th Edn., Ames: Iowa State Press, pp 739 -758.

Goto, Y., Nakura, R., Nasu, T., Sawada, T. and Shinjo, T. (2001). Isolation of *Pasteurella multocida.* During an outbreak of infectious septicaemia in Japanese quail (*Coturnix coturnix Japonica*). *Journal of Veterinary Medical Science*, **63**(9): 1055-1056.

Haruna, E.S., Musa, U., Okewole, P.A., Shamaki, D., Lombin, L.H., Molokwu, J., Edache, J.A. and Karsin, P.D. (1997). Protein requirement of quail chicks in Plateau State, Nigeria. *Nigeria Veterinary Journal*, **18**: 108-113.

Hinshaw, W.R. and Emlen, J.T. (1943). *Pasteurellosis* in California Valley quail. *The Cornell Veterinarian*, **33**: 351-353.

Kumar, A.A., Shivachandra, S.B., Biswas, A., Singh, V.P., Vijendra, P. and Srivastava, S.K. (2004). Prevalent serotypes of *Pasteurella multocida* isolated from different animals and avian species in India. *Veterinary Research Communication*, **28**: 657- 667.

Miguel, B., Wang, C., Maslin, W.R., Keirs, R.W. and Glisson, J.R. (1998). Subacute to chronic fowl cholera in a flock of Pharaoh Breeder quail. *Avian Diseases*, **42**: 204 - 208.

National Research Council (NRC) (1991). The quail bird. Board for Science and Technology for International Development, Academic Press, Washington, D. C. pp 147-155.

Odugbo, M.O., Muhammad, M., Musa, U., Suleiman, A.B., Ekundayo, S.O. and Ogunjumo, S.O. (2004). *Pasteurellosis* in Japanese quail (*Coturnix coturnix japonica*) caused by *Pasteurella multocida* A: 4. *Veterinary Record*, **155**: 90 - 91.

Oladele, S.B., Enoch, I., Lawal, S. and Ibu, O.J. (2008). Clinicopathological features of Newcastle disease in Japanese quails

(*Coturnix coturnix japonica*) infected with Newcastle disease virus Kudu 113 strain. *International Journal of Poultry Science*, **7**: 165-168.

Quinn, P.J., Markey, B.K., Leonard, F.C., Fitz Patrick, E.S. and Fanning, S. (2011). *Veterinary Microbiology and Microbial Diseases*, 2nd Edn. Wiley-Blackwell. Ames, Iowa, USA, pp 300 -308.

Raji, M.A., Ahmed, J.S., Saidu, L. and Ameh, J.A. (2010). Retrospective studies on the prevalence of fowl cholera in Zaria Kaduna State Nigeria. *Sokoto Journal of Veterinary Science*, **8**(1): 9 -11.

Rhoades, K.R. and Rimler, R.B. (1990). *Pasteurella multocida* colonization and invasion in experimentally exposed turkey poults. *Avian Diseases*, **34**: 381-383.

Shilpa, S. and Verma, P. C. (2006). Pathology of *Pasteurella multocida* infection in chicken. *Indian Journal of Animal Research*, **40**: 15-19.

Shilpa, V.T., Girish, B.C., Ambujakshi, T.P. and Manjunatha, K.P. (2015). An outbreak of Avian Pasteurellosis in a turkey farm. *International Journal of research – Granthaalayah*, **3**(11): 43 - 46.

Townsend, K.M., Frost, A.J., Lee, C.W., Papadimitriou, J.M. and Dawkins, H.J.S. (1998). Development of PCR assays for species and type specific identification of *Pasteurella multocida* isolates. *Journal of Clinical Microbiology*, **36**: 1096 -1100.

Yakubu, D., Moshood, R., Paul, A., Sunday, O. and Lola, O.M. (2015). Clinicopathological features in Japanese quails (*Coturnix coturnix japonica*) inoculated with *Pasteurella multocida* serotypes A: 1, 3 and 4. *World's Veterinary Journal*, **5**(2): 26 -30.