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Research Article

AN OUTBREAK OF PASTEURELLOSIS IN JAPANESE QUAIL CHICKS (*COTURNIX COTURNIX JAPONICA*)

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Avian pasteurellosis is a contagious disease of domestic and wild birds caused by *Pasteurella multocida*. A private broiler quail farm (*n*=1100) with a mortality of 330 eight day old quail chicks without clinical signs were presented to the Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu, Tamilnadu, India. On complete post-mortem examination, visceral organs showed generalised congestion and consolidation of lung. Histopathologically, liver showed severe congestion, multifocal necrotic hepatitis with heterophilic infiltrations. Lungs revealed bronchopneumonia. Cytological examination of lung impression showed bipolar organisms. Microbiological examination with heart swab, lung and liver tissue revealed *Pasteurella* sp. The bacteria were confirmed by PCR assay with KMT1 gene as *Pasteurella multocida*. Based on history, gross, cytology, microbiological and histopathological lesions the case was diagnosed as avian pasteurellosis.

Key words: Japanese quail, Pasteurellosis, Pathology, Polymerase chain reaction

Fowl cholera is a contagious bacterial disease of domesticated and wild avian species caused by infection with *Pasteurella multocida* which hamper the profitable poultry production (Raji *et al.*, 2010). It usually appears as a septicemic disease often associated with high morbidity and mortality, but chronic or benign conditions may also occur (Glisson *et al.*, 2008). It affects all type of birds.

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Among domestic birds, turkeys tend to be more susceptible followed by chicken and ducks (Glisson *et al*., 2008; Quinn *et al*., 2011; Shilpa *et al*., 2015). Fowl cholera generally occurs between 10 to 13 weeks of age and rarely in birds less than 2 weeks of age (Glisson *et al*., 2008). NRC (1991) reported that young birds and quails are resistant to the infection by *Pasteurella multocida*. Obvious clinical signs of acute fowl cholera may not occur until very late in the infection and include depression, ruffled feathers, fever, anorexia, mucous discharge from the mouth, diarrhoea, respiratory distress (Rhoades and Rimler, 1990). Cyanosis of comb and wattle with mortality may reach 0 to 20% in chicken (Glisson *et al*., 2008). Grossly, the affected dead birds may show extensive congestion of visceral organs with necrotic hepatitis, congestion and hemorrhages in intestinal mucosa, petechial hemorrhages on the pericardium with serofibrinous pericarditis (Sharma *et al*., 1974; Glisson *et al*., 2008).

There were extensive literatures on the incidence, prevalence, clinical manifestations of fowl cholera in chickens, turkeys, ducks, geese (Glisson *et al*., 2008) and emu (Anitha and Mammen, 2013) but only a few reports in quail species. This study documents the occurrence of acute fowl cholera in 8 days old Japanese quail chicks.

MATERIALS AND METHODS

A private broiler farm (n=1100) at Thanjavur reported 330 deaths of Japanese quail chicks with the history of sudden death in a span of seven days. Quail chicks were kept in brooding cage along with broiler chicks brooding cages. Birds were not vaccinated against any viral infections and not treated with drugs. The chicks were kept close to the grower flocks which suffered fowl cholera recently. A few dead quail chicks were brought to the Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamilnadu, 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 (Buxton and Fraser, 1977; Quinn *et al*., 2011). Impression smears from lungs and heart blood were collected for cytological study. Representative tissue samples of lungs and liver were collected from all the quail chicks in 10% formalin for histopathological studies and without preservative for carrying out PCR assay. PCR assay was carried out for KMT1 gene with KMT1T7 and KMT1SP6 primers (Townsend *et al*., 1998). Primer sequences as

KMT1T7 5'-ATC-CGC-TAT-TTA-CCC-AGT-GG-3'

KMT1SP6 5'-GCT-GTA-AAC-GAA-CTC-GCC-AC-3'

Touch impression smears were stained with Grams and Leishman's staining technique. Tissue samples collected during necropsy

in 10% formalin were processed as per standard paraffin embedding technique, sectioned at 3µ thicknesses and stained with routine haematoxylin and eosin method (Bancroft and Gamble, 2008).

RESULTS

Mortality was reported to be increasing from second day to eight day with a total mortality of 330 chicks (Fig.1). On external examination, chicks appeared thin with pale mucous membrane. Grossly, visceral organs revealed slight to moderate congestion. Duodenum was congested. Lungs were dark brownish and firm. Liver was severely congested and/or mottled.

The impression smears of lung and heart blood smears revealed numerous, small, Gram negative, typically bipolar organisms (Fig. 2).

Histopathological studies revealed diffuse mild congestion in all visceral organs. Liver showed moderate congestion, diffuse vacuolar changes of hepatocytes and multifocal coagulative necrosis with heterophilic infiltrations (Fig. 3). Lungs revealed multifocal subacute broncho-pneumonia (Fig. 4).

The organisms from swabs of lung and 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. 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.

Polymerase chain reaction (PCR) revealed amplification of KMT1 gene with 460 bp in all isolates which is consistent with *Pasteurella multocida* (Fig. 5).

DISCUSSION

Fowl cholera in quails was first reported by Hinshaw and Emlen (1943). Chicken less than 16 weeks of age and quails were reported to be resistant to fowl cholera as per NRC (1991), whereas in the present case fowl cholera occurred in 8 day old quail chicks. Mortality can vary from 60% (Myint and Carter, 1988; Miguel *et al*., 1998) to 99% (Bermudez *et al*., 1997) in natural outbreaks of fowl cholera in quails. In this study, mortality in the Japanese quails was first noticed from second day of hatch. High mortality of about 30% at 8 days recorded in this study was in concordance with the earlier research reports (Myint and Carter, 1988; Bermudez *et al*., 1997; Miguel *et al*., 1998).

The quail chicks which died without any premonitory 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 gross lesions observed in this study were similar with the earlier findings

(Bermudez *et al*., 1997; Glisson *et al*., 1989; Goto *et al*., 2001; Oladele *et al*., 2008; Akpavi *et al*., 2011) in quails. Grossly, pale necrotic spots in liver of chicken affected with fowl cholera was lacking in this present study. Absence of remarkable hepatic lesions was also recorded previously (Glisson *et al*., 1989) in 24-28 days Japanese quail naturally infected with *Pasteurella multocida.* Histopathologically, liver with multifocal coagulative necrosis and heterophilic infiltrations pathognomonic to avian pastuerellosis observed in this study coincided with the findings of others (Naveen and Arun, 1992; Glisson *et al*., 2008; Yakubu *et al*., 2015). This study concluded that the gross lesions were not marked in 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), first time the present study described the subacute bronchopneumonia in acute pastuerellosis infection in 8 day old Japanese quail chicks which was in-accordance with the earlier report of Shilpa *et al*. (2015) in turkey.

The PCR assay with 460bp product was in consistant 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 quail brooding cages were kept with the broiler cages and 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 chicks more vulnerable for the establishment of *Pasteurella multocida* within a day.

This present study concludes that the infection with *Pasteurella multocida* can occur even within 8 days after hatching in Japanese quails when remarkable predisposing factors coexist. It also records that *Pasteurella multocida* infection in Japanese quails leads to severe bronchopneumonia on par with turkeys.

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Pasteurellosis in Japanese quail chicks

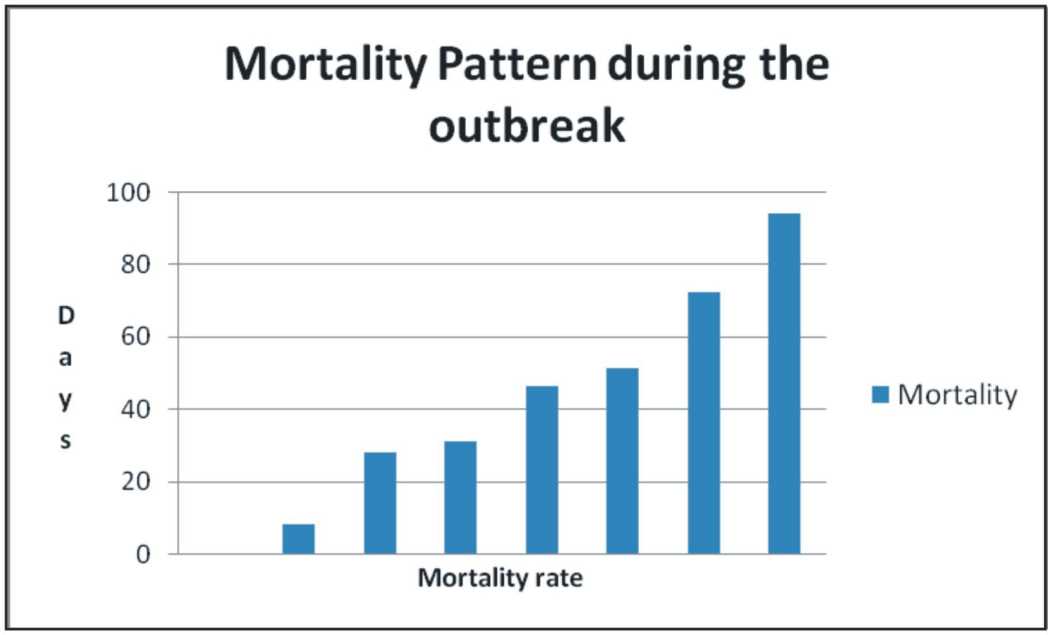

Fig. 1. Mortality pattern of Japanese quails in an outbreak with *Pasteurella multocida*

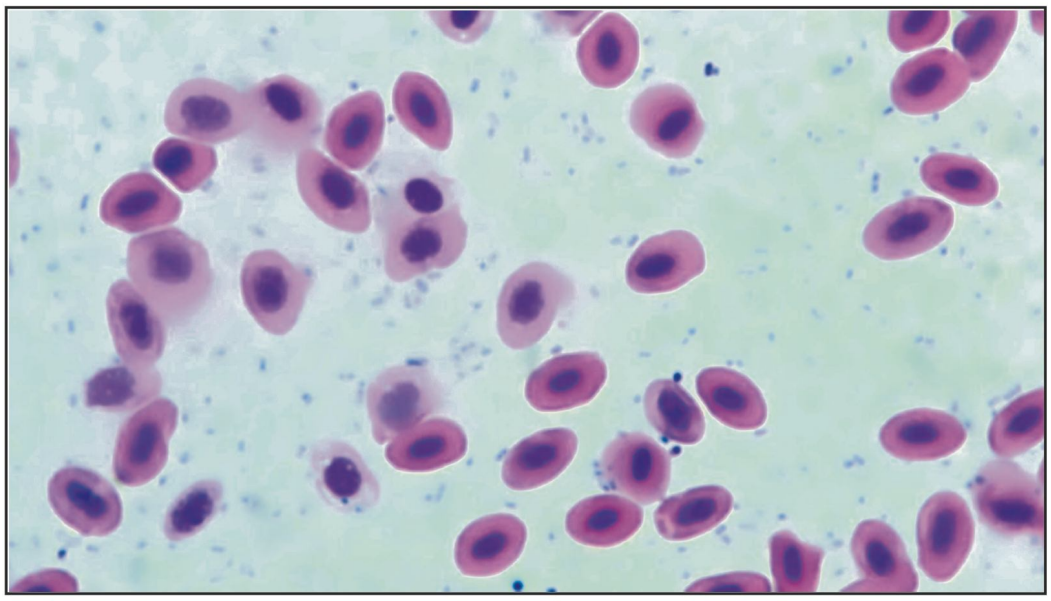

Fig. 2. Scattered numerous, small, typically bipolar organisms- impression smear-lung Leishman stain x 1000

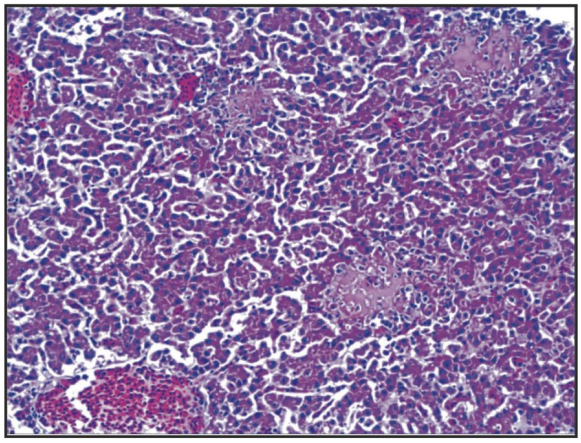

Fig. 3. Multifocal coagulative necrosis of hepatocytes with heterophilic infiltration of liver in Japanese quail -H&E x 400

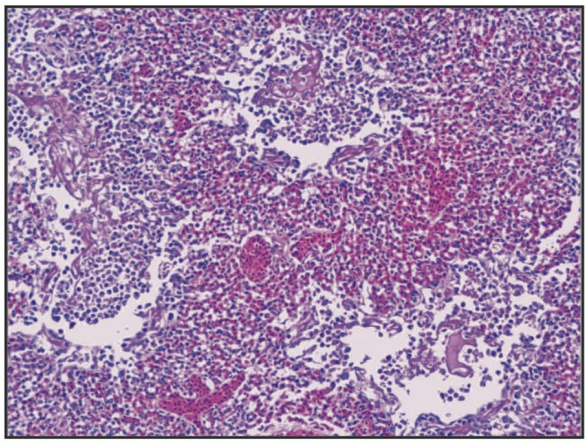

Fig. 4. Multifocal bronchopneumonia of lung in Japanese quail- H&E x 400

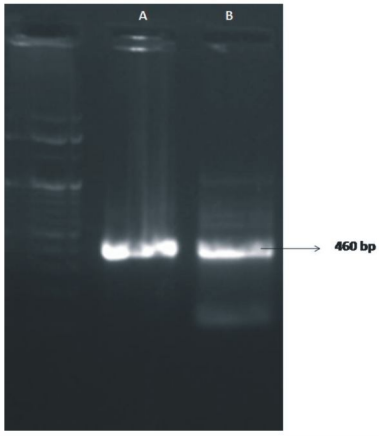

Fig. 5. Polymerase chain reaction of (A) lung tissue and (B) liver tissue for *Pasteurella multocida*

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