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CLINICOPATHOLOGICAL CHANGES OF EXPERIMENTALLY INDUCED FOWL CHOLERA IN JINDING DUCKS

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ABSTRACT

Clinicopathological changes of duck cholera were studied in 16-week-old 10 susceptible (PHA titre ≤ 8.00) Jinding breed ducks during the period from October 2002 to March 2003. Each of the experimental duck was inoculated with a virulent chicken isolate of *Pasteurella multocida* @ 5.4 x 10^6 CFU / ml per bird intramuscularly. The incubation period varied from 12 to 48 hours with 100% mortality rate between 24 to 72 hours of infection. These affected ducks showed clinical signs of fever, anorexia, dullness, depression, ruffled feathers, severe weakness, drowsiness, whitish diarrhoea with mucus, exudation from eyes, lameness and unable to hold the head with beak touching the ground before death. Necropsy examination of the dead ducks showed septicaemic conditions, blood vascular congestion, haemorrhagic enteritis, increased amount of pericardial and peritoneal fluids, swollen and sometimes congested liver with multiple necrotic foci on the parietal surface, enlarged and edematous heart with echymotic haemorrhages. Trachea and lungs were severely congested and haemorrhagic and serofibrinous exudates were seen in the lung, liver and heart. The *P. multocida* organism was reisolated from the swabs of liver and heart of all the dead ducks by standard bacteriological techniques. This study was confirmed the virulence and pathogenicity of chicken isolate of *P. multocida* in ducks.

Key words: Experimental infection, Duck cholera, pathogenicity, clinicopathological changes

INTRODUCTION

Fowl cholera is one of the most important and devastating endemic avian disease in Bangladesh (Chowdhury *et al.*, 1985 ; Baki *et al.*, 1993). Israil and Quader, 1967) first isolated *Pasteurella multocida* from a case of Muscovy duck and subsequently Ali (1974) isolated *P. multocida* from two ducks and described the disease condition as Duck cholera. Baki *et al.* (1993) reported proportionate mortality rates of 54.55% in chickens and 10.91% in ducks, caused by Fowl cholera in Bangladesh. Although some reports on experimental studies on Fowl cholera in chickens have been made (Kamal *et al.*, 1988 ; Khan *et al.*, 1997 ; Hossain *et al.*, 1999) but similar reports are very limited in ducks in Bangladesh (Baki *et al.*, 1991). This paper describes the pathogenicity and clinico-necropsy changes of experimentally induced in Jinding ducks with a virulent chicken isolate of *P. multocida*.

MATERIALS AND METHODS

Four-week-old 50 Jinding breed of ducks were purchased from the Government Poultry Farm, Kishoregonj, of which only 10 birds were randomly selected for experimental production of Fowl cholera at the age of 16 weeks. These birds of both sex with no previous history of either vaccination or Fowl cholera infection (specific pathogens free) were selected for this study. These ducks were maintained in the newly constructed duck house in the Department of Veterinary Medicine, Bangladesh Agricultural University (BAU), Mymensingh with intensive care, adequate commercial feed (Quality Feeds Ltd., Dhaka) and water supply throughout the experimental period. In addition to general feed supply, vitamin-mineral premix (Megavit®, Novartis Bangladesh Ltd.) was also supplied in the drinking water daily.

Venous blood samples were collected from the wing vein of each of the 10 ducks at the age of 16 weeks before infection and sera were separated as per standard conventional method and tested by the Passive haemagglutination assay (PHA) to detect the level of maternal derived antibodies as described by Tripathy *et al.* (1970). The detected antibody titre ranged from 4.0 to 8.0 with an average of 5.6 ± 1.96 (SD) against the protective level of ≥ 1 : 64.

A local virulent chicken isolate of *P. multocida* (PM-38), serotypes-1 (X-73), preserved in laboratory at room temperature in mineral oil was used for experimental infection in this study. The virulence of the preserved bacterial stock was reverted by serial culture on blood agar as described by Chowdhury *et al.* (1987), Khan *et al.* (1997) and Hossain *et al.* (1999). Accordingly, bacterial inoculum containing approximately 5.4 x 10^6 CFU / ml in PBS was prepared by standard dilution method to produce experimental infection in ducks.

Each of the 10 experimental ducks was intramuscularly inoculated with 1.0 ml of 5 x 10^6 CFU / ml live virulent *P. multocida* organism at the age of 16 weeks. All the experimentally infected ducks were observed daily to record the signs and symptoms up to the time of death.

Necropsy and bacterial isolation

Necropsy examination was performed as soon as the experimentally infected ducks were found dead. Swabs or tissue materials from liver and heart were streaked onto blood agar plates. The plates were incubated at 37° C for 24 hours to detect the growth of organisms. The positive cases showing growth of *P. multocida* were confirmed by standard bacteriological procedure as described by Cowan and Steel (1985).

RESULTS AND DISCUSSION

A virulent chicken isolate of *P. multocida* (5.4 x 10^6 CFU / ml) was used for experimental production of infection in Jinding breed of duck @ 1.0 ml / bird intramuscularly to study its pathogenicity and clinicopathologic changes. The incubation period of intramuscular inoculation of *P. multocida* in 10 Jinding ducks varied from 12 to 48 hours, of which 12 hours recorded in 4 (40%), 24 hours in 5 (50%) and 48 hours in 1 (10%) duck. This observation supports the earlier reports of Baki *et al.* (1991) who reported 18 to 24 hours incubation period in ducks inoculated with 4 x 10^6 CFU / bird intramuscularly, and Khan *et al.* (1997) who reported 10 to 12 hours incubation period in chickens inoculated intramuscularly with 5.0 x 10^6 CFU / ml *P. multocida* organisms. These findings of incubation period in this study also support the reports of Rhoades and Rimler (1990) who detected the *P. multocida* that colonies in the dorsal pharynx within 6 hours of experimental infection and then invade the blood system within 6 to 12 hours of infection. Tsuji and Matsmoto (1989) reported that once bacteria entered into the circulation they were trapped in liver and spleen and phagocytised there. The capsulated bacteria easily escaped from the phagocytic process and produced disease rapidly (Snipes *et al.*, 1987).

The clinical signs first observed at 12 hours of post-infection (PI) that include dullness and depression, slight rise of body temperature (41.5 ^{0}C) and ruffled feathers (Fig. 1). At 24 hours PI, ducks showed the signs of severe weakness, drowsiness, anorexia, fever (43.5 ^{0}C), exudation from eyes (Fig. 2), lameness, whitish diarrhoea with mucus and unable to hold the head (Fig. 3) with head touching the ground before death was noticed in some ducks. At 48 hours of PI, there was slight cyanosis characterized by dark red coloured wattles, comb and ear lobes. The other signs included anorexia, lameness, greenish diarrhoea with mucus and subnormal cloacal temperature (40.5 ^{0}C). Laboured breathing, emaciation and dehydration, followed by moribund and death (Fig. 4). Death of four ducks recorded at 24 hours, three at 48 hours and other three died at 72 hours PI. Death of ducks due to *P. multocida* infection might be caused by endotoxic shock (Park, 1982 ; Lee *et al.*, 1992). However, Hossain *et al.* (1999) suggested that neural degeneration, cardiomyopathy, severe pneumonia, extensive lesions in liver, blood loss due to haemorrhages, dehydration and inanition along or in combination may have also contributed to the clinical manifestations and death of birds.

The presence of lesions in the liver and kidneys in Fowl cholera might have resulted defective metabolism of uric acid and urates and the development of whitish diarrhoea might be related to the presence of uric acid and urates in the faeces (Khan *et al.*, 1994 ; Hossain *et al.*, 1999). The occurrence of leg weakness, immobility and lameness in Duck cholera might be due to arthrosynovitis and tenosynovitis (Karim, 1987). Laboured breathing and dyspnea might be due to exudation and edema of the lungs (Hossain *et al.*, 1999). The clinical findings of Fowl cholera recorded in Jinding ducks in this study are in conformity with the earlier reports made on ducks (Baki *et al.*, 1991) and chickens (Khan *et al.*, 1997 ; Hossain *et al.*, 1999).

All the 10 ducks experimentally infected with *P. multocida* died between 24 to 72 hours post-infection and necropsy examination of these ducks showed severe septicaemic conditions with blood vascular congestion (Fig.5) and haemorrhages throughout the internal organs with characteristics in the lung, abdominal fat and intestinal mucosa (Fig. 6) and occasionally 'pinpoint' haemorrhage in the breast muscles with increased pericardial fluid and echymotic haemorrhage in the heart (Fig. 7) and peritoneal fluid. Swollen and congested liver with multiple necrotic foci on the parietal surface (Fig.8), and enlarged and edematous heart (Fig. 9) were recorded. Trachea and lungs were haemorrhagic (Fig.10) and contained increased amount of secretary fluid and viscid mucus in the pharynx, crop and intestine. These necropsy findings of Fowl cholera in ducks are in conformity with the earlier reports made on ducks (Karim, 1987 ; Baki *et al.*, 1991, 1993) and chickens (Kamal *et al.*, 1988 ; Khan *et al.*, 1997).

Bacteriological isolation from dead ducks

Swabs were collected from the liver and heart of all the 10 dead ducks and streaked onto blood agar plates, and then the plates were incubated at 37 ^{0}C for 24 to 48 hours. All the swabs cultured from 10 ducks showed growth of *P. multocida* in the inoculated plates and were further confirmed by standard bacteriological procedure (Cowan and Steel, 1985). Briefly, the growth of *P. multocida* organisms in blood agar yielded small colonies with whitish opaque, circular and translucent appearance and no haemolysis (Fig. 11).

Fig. 1. A 16-week-old Jinding duck experimentally infected with Duck cholera showing clinical sings of dullness, depression and ruffled feather at 12 hours of post-infection.

Fig. 2. Experimentally produced Duck cholera in a 16-week-old Jinding duck showing exudation from eyes with matted feathers at 24 hours of post-infection.

Fig. 3. Experimentally induced Duck cholera in a 16-week-old Jinding duck showing characterstic whitish diarrhoea and unable to hold the beak at 24 hours of post-infection.

Fig. 4. Photograph of three Jinding ducks, of which left two are dead and right one at moribund stage at 24 hours of post-experimental infection with virulent *Pasteurella multocida*.

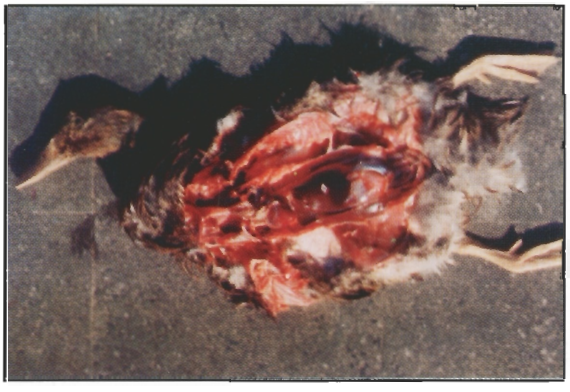

Fig. 5. Post-mortem lesions of a duck died of experimental Duck cholera showing severe congestion and haemorrhage of almost all the internal organs.

Fig. 6. A portion of a intestine of a duck died of experimental Duck cholera showing extensive congestion and haemorrhage of the intestinal mucosa.

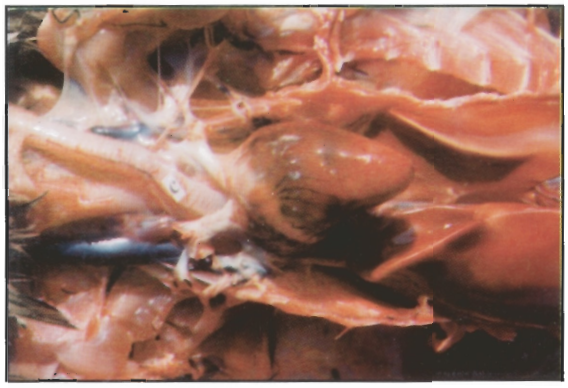

Fig. 7. Necropsy changes of a duck died due to an experimental Duck cholera showing echymotic haemorrhages in the heart.

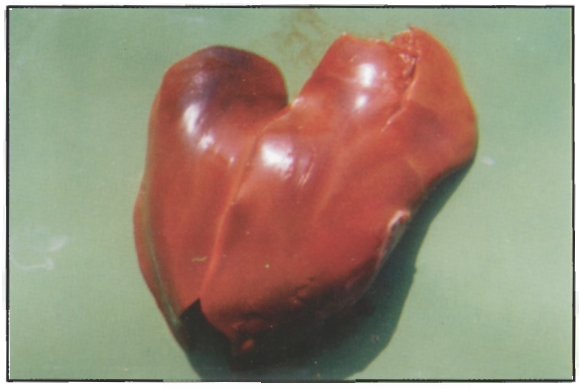

Fig. 8. A liver of a duck died due to experimental Duck cholera showing swollen and congested the whole liver.

Fig. 9. A heart of duck died due to experimental Duck cholera showing congestion and sub-epicardial haemorrhages.

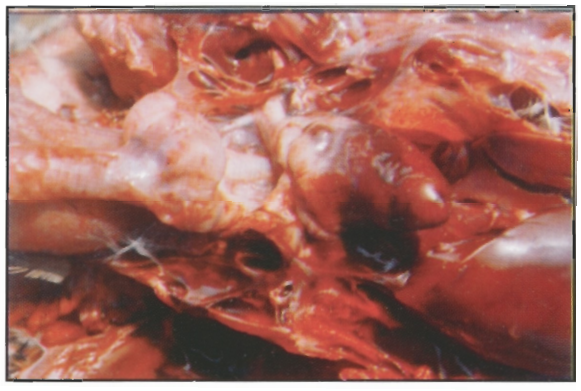

Fig. 10. Post-morten changes in duck died due to experimental Duck cholera showing congested and haemorrhagic trachea, lungs, liver and heart.

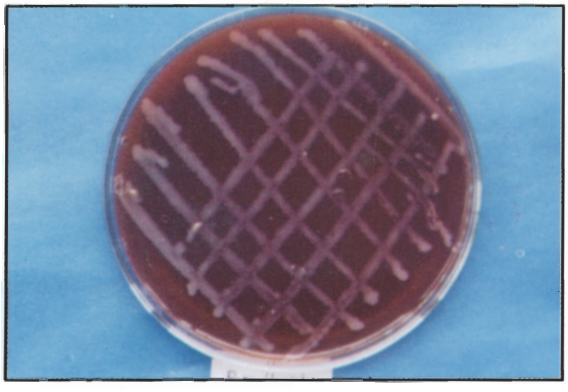

Fig. 11. A pure culture colony of *Pasteurella multocida* on blood agar isolated from a duck died of experimental Fowl cholera infection.

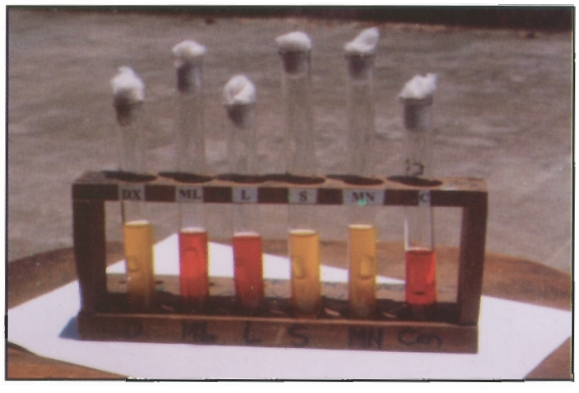

Fig. 12. Results of biochemical test of *P. multocida* isolated from dead duck due to experimental Fowl cholera showing fermentation of dextrose, sucrose and mannitol but not fermented lactose and maltose.

Gram's staining of the impression smears of heart and liver, and smears prepared from culture materials revealed Gram's negative, cocco-bacillary and bipolar organisms. Biochemical tests of the isolated organisms showed complete fermentation of dextrose, sucrose and mannitol but did not ferment lactose and maltose (Fig. 12) which are the characteristics of *P. multocida* organisms (Calnek *et al.*, 1997).

It appears from the results of this study that *P. multocida* isolated from chickens is also highly pathogenic which caused 100% mortality to ducks with same dose (5.4 x 10^6 CFU / ml) of inoculum.

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