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Research Article

Isolation and molecular confirmation of *Pasteurella multocida* from acute infection in Khaki Campbell ducks

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Abstract

Avian pasteurellosis is a contagious disease of domestic and wild birds caused by *Pasteurella multocida*. Acute duck cholera is characterized by severe watery diarrhea, anorexia, respiratory manifestations and septicemia, while chronic one leads to localization of the organism in certain organs. Thus, the present investigation was conceptualized to detect one of the virulent pathogens affecting duck population by gross pathology and PCR in and around Gazole block, Malda, West Bengal. *Pasteurella multocida* was isolated from the collected samples and confirmed by KMT-gene specific PCR. This investigation suggests that molecular detection of the bacteria is a useful and rapid technique to apply to field samples.

Keywords: Duck Cholera, *Pasteurella multocida*, PCR, West Bengal

Highlights

- Duck cholera is a major infection, caused by *Pasteurella multocida* associated with high mortality.
- In an outbreak in Khaki Campbell duck, gross pathology was suggestive of duck cholera.
- *Pasteurella multocida* was isolated and identified by biochemical tests.
- *Pasteurella multocida* was confirmed by KMT-PCR.

INTRODUCTION

Duck farming is one of the major tools for income generation in the rural economy due to the high prolificity and easily adaptable nature of the ducks to free-range system with simple needs of housing (Rajput *et al*., 2014). West Bengal, Assam, Tamil Nadu and Kerala are major duck rearing states in India. The occurrence of infectious diseases is considered to create hindrances in the profit generation process of duck rearing. Duck cholera is one of the major bacterial infections caused by *Pasteurella multocida*, which is responsible for high mortality (50%) in ducks. Prevalence of *P. multocida* carriers in healthy duck flocks is 10% (Eid *et al.*, 2019). Proper diagnosis of infections is the prerequisite for effective treatment and prevention of the infection.

P. multocida is a Gram negative, non-motile, non-spore forming rod-shaped organism that occurs in single or pairs or occasionally as chain forms or filaments. A capsule can be demonstrated using an indirect method of staining of freshly cultured bacteria (Swayne, 2013). Infection with virulent *P. multocida* strains usually induces gross lesions in the liver, spleens and other viscera, resulting in the death of birds in several days. *P. multocida* isolates are classified into five capsular serogroups (A, B, D, E & F) and 16 Heddleston lipo-polysaccharide (LPS) serovars (Zhao *et al*., 2022). They can currently also be divided into 8 LPS genotypes (L1-L8) according to the LPS outer core gene cluster (Harper *et al*., 2015).

The traditional method of diagnosing bacterial disease is by culturing bacteria on agar plates followed by biochemical and serological testing of the isolates, which is time-consuming, tedious and shows less sensitivity (Wei *et al*., 2013). Molecular techniques such as PCR are a highly sensitive way to detect specific pathogens in field samples (Xiao *et al.*, 2021). Thus, the present investigation was conceptualized to detect one of the virulent pathogens affecting duck population by gross pathology and PCR in and around Gazole block, Malda, West Bengal.

MATERIALS AND METHODS

Sample collection: Around 500 ducks aged from two (02) to six (06) months were examined during the month of June 2021. Sudden mortality with a history of dullness, depression and mucous discharge from the mouth was observed in the flock during the specified time. On post-mortem examination, the following gross lesions were observed.

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a) Trachea- Congested with blood clot inside the tracheal lumen
b) Lung- Haemorrhagic and consolidated
c) Thoracic cavity- Petechial to extravasations inside the wall of the thoracic cavity
d) Heart- Haemorrhage over the pericardial surface of the heart with straw colour pericardial fluid
e) Liver- Congested with focal necrosis, fragile
f) Intestine- Congested

Liver and heart samples were collected from dead birds. For microbiological examination and confirmatory identification of the causative organism, the samples were immediately transported to the Institute of Animal Health and Veterinary Biologicals, Kolkata, West Bengal.

Isolation of *Pasteurella multocida* from collected samples

An impression smear was prepared from the collected liver sample and was stained with methylene blue. The collected samples were plated on blood agar (HiMedia, India) and MacConkey agar (HiMedia, India). All plates were incubated at 37°C for a minimum of 48 hours. Preliminary identification of *P. multocida* isolates was carried out according to colony characteristics, Gram-staining, and standard biochemical tests such as catalase, oxidase, indole, citrate, urease and TSI agar tests (Quinn *et al*., 1994).

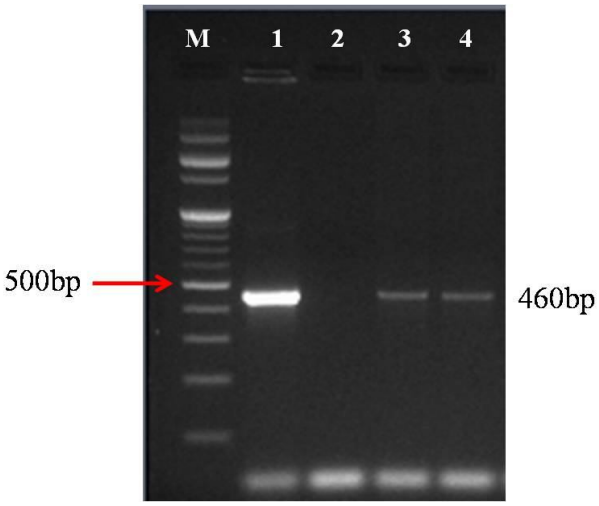
Genomic DNA extraction: DNA was extracted from the suspected colony by heatlysis method. The 'dew drop' colonies in blood agar, preliminarily identified by Gram's stain and biochemical tests, were suspended into 1.5 mL of microcentrifuge tube containing 50 µL of molecular grade nuclease free water (HiMedia, India). The tube was placed in a boiling water bath for 10 minutes, followed by snap chilling and centrifugation at 13000 rpm for 5 minutes. Without disturbing the pellet, the supernatant was removed and stored at -20°C until further analysis.

Polymerase chain reaction (PCR): Polymerase chain reaction was performed using *P. multocida* species-specific primers KMT ISP 6 (5'- ATCCGCTATTTACCC AGTGG- 3') and KMT 177 (5'- GCTGTAAACGAACT CGCCAC- 3') designed by Townsend *et al*. (1998) to amplify KMT1gene. The PCR protocol was followed as 95°C for 15 minutes; 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds and final extension of 72°C for 10 minutes. The PCR amplicons with positive and negative controls were checked by electrophoresis on 1.5% low melting agarose gel stained with ethidium bromide (0.5 µg/mL) and documented under Gel documentation system (BioRad, USA). *Pasteurella multocida* (ATCC 43137) and *Escherichia coli* (ATCC 25922) served as positive and negative controls, respectively.

RESULTS

The clinical signs of acute illness followed by sudden death in the ducks aged between 2-6 months and gross pathology of vascular damage and focal necrosis in the liver and other vital organs were suggestive of acute duck cholera infection (Fig. 1,2,3). For confirmation of the infection, laboratory diagnosis was required. Impression smear of the liver showed typical bipolar organisms.

In blood agar, non-haemolytic, smooth 'dew drop' like colonies were observed after 48 hours of incubation. No growth was detected in MacConkey agar. The isolates were Gram-negative coccobacilli, catalase positive and positive for indole production. All the isolates were negative for urease production. The isolates were confirmed by PCR as *Pasteurella multocida* due to the production of the specified amplicon (460 bp, Fig. 4).



[M- 100 bp plus ladder; Lane 1- Positive Control (*Pasturella multocida*); Lane 2- Negative Control; Lane 3, 4- Representative samples from duck]

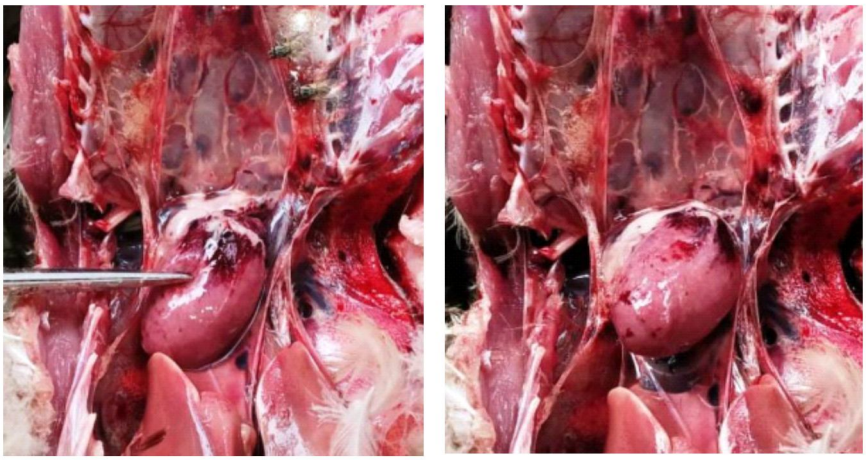
Fig. 4. PCR amplification of KMT1 gene of *P. multocida* in 1.5% low melting agarose gel

DISCUSSION

Ducks constitute a major component of the poultry industry throughout the world. In India, the 20th Livestock Census identified 33.51 million ducks, which was 23.53 million during the 19th Census (DAHD, 2019). The substantial increase (42.3%) in duck population indicates high demand for duck farming, which deserves proper surveillance and monitoring of the infections to formulate the control and prevention strategy. West Bengal and Kerala are the major duck rearing states with a high consumption rate of duck meat and eggs that meets the requirement for taste of the consumers developed on fish associated food (Rajput *et al*., 2014).

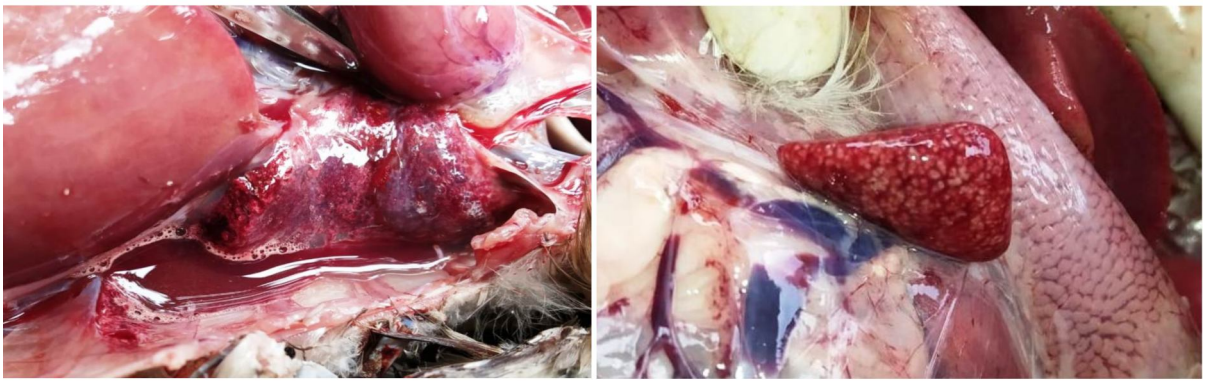
The present study reported sudden mortality of Khaki Campbell ducks (2-6 months old) with a history of

Pasteurella multocida infection in Khaki Campbell duck



(a) (b)

Fig. 1 (a-b). Pericardial haemorrhages in the heart of duck



(a) (b)

Fig. 2 (a-b). Focal necrosis on the surface of liver in duck

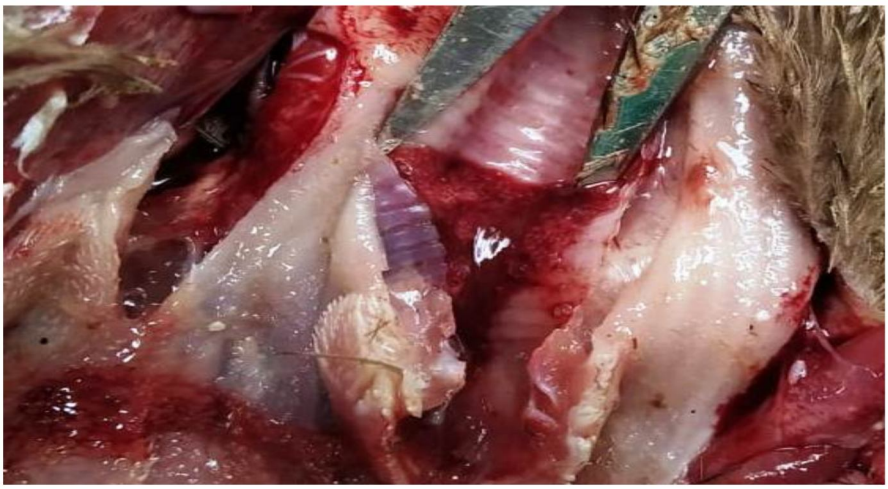


Fig. 3(a-b). Haemorrhages in intestinal mucosa in duck

dullness, depression and mucous discharge from the mouth in Malda, West Bengal. The clinical symptoms, mortality rate and the susceptible age of the ducks were suggestive of acute duck cholera infection (Islam *et al*., 2003). Gross pathology like widespread vascular damage, focal necrosis on the surface of the liver and other vital organs, edema and congestion in lungs, haemorrhage in the intestinal mucosa, and pericardial haemorrhages were also suggestive of duck cholera infection as observed in earlier reports (Hunter and Wobeser, 1980; Punnoose *et al*., 2021). Finally, the infection was confirmed as duck cholera with the isolation and KMT-PCR based confirmation of *P. multocida.* However, the capsular types of *P. multocida* isolates could not be confirmed, which is a drawback of the present study.

Duck cholera is prevalent throughout the world, especially in Asia and Middle East countries (Punnoose *et al*., 2021). In Bangladesh, the prevalence of duck cholera was estimated as 11% (95%-CI:2-25%) (Patil *et al*., 2021). Similar to the present finding, duck cholera infection was reported earlier in different parts of India (Devi *et al*., 2000; Bhattacharya, 2005), including West Bengal (Das *et al*., 1991; Sadhukhan *et al*., 2006; Maity *et al*., 2012). Infection in ducks generally results during the entry of *P. multocida* into the tissues of birds through the pharynx or upper respiratory tract, eye membrane and injuries in the skin. The infection is characterized by rapid onset and high mortality rate, as observed in the present study. The period of the infection showing clinical symptoms is brief, although few symptoms like lethargy, convulsions, throwing their head back between the wings, soiling feathers around the vent, eyes and yellow-coloured droppings are exclusive to duck cholera and were detected in the present study.

Conflict of interest: There is no conflict of interest regarding the present research work.

Author's contribution: SKM, SG: Conducted the gross pathology and collected the samples; DP: Conducted isolation and PCR work; IS: Conceptualized the study and wrote the manuscript.

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