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Scientific Report

Duck plague outbreak in a Chara-Chemballi duck farm

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Abstract

Background: Duck rearing is one of the important livelihoods of rural people. Duck plague is one of the diseases causing heavy mortality resulting in economic losses. **Case description:** An outbreak of duck plague in a farm in Kadavakathi Village near Tenkasi, Tirunelveli Dt., is reported. **Findings/treatment and outcome:** Two thousands out of 4500 Chara-Chemballi breed of ducks which were recently purchased from Chenganacherry in Kerala died, with a mortality rate of 44.4%. Clinical signs of inappetence, partial closure of eyelid, conjunctivitis, corneal opacity, oculo-nasal discharge, soiled vent with green white watery diarrhoea, ataxia, incoordination and sudden death were observed. Necropsy examination revealed diphtheritic membrane in the oesophagus, congestion, petechial haemorrhages and multifocal gray white areas on the surface of the liver, epicardial haemorrhages, congested trachea, lung, kidneys, splenomegaly with mottled appearance and enteritis. Microscopical examination revealed presence of eosinophilic intranuclear and intracytoplasmic inclusions in the epithelial cells of the intestine and hepatocytes, degeneration and necrosis of enterocytes, dilated crypt epithelial cells with presence of eosinophilic intranuclear and intracytoplasmic inclusions, congestion and lymphoid cell depletion in the spleen, vasculitis, congestion, and haemorrhages in the trachea and lungs, proventriculitis, and congested kidneys. Polymerase chain reaction (PCR) also confirmed the duck plague viral infection by the amplification of polymerase gene fragment (446 bp). **Conclusion:** Based on the above findings, the Chara-Chemballi duck disease outbreak was diagnosed as duck viral enteritis infection.

Key words: Chara-Chemballi duck, Duck plague, Molecular identification, Pathology

Introduction

Duck husbandry is important in the upliftment of downtrodden rural people of Tamil Nadu State, India. Chara-Chemballi is one of the indigenous duck breeds in Kerala State of India. Chara-Chemballi breed population was 1,45,237 as per 2012 census and the breed has superior traits in egg weight and egg production. Duck plague is an acute, highly contagious and fatal disease of domestic ducks and water fowl (Kaleta *et al*., 2007). The first report of duck plague recorded in Netherlands in 1923 and later from other countries (Wang *et al*., 2013). The morbidity and mortality percentage caused by duck plague varies from 5 to 100% (Sandhu and Metwally, 2008). It is usually spread by close contact under good conditions (Kaleta *et al*., 2007). During the period of outbreak, infected birds act as a source to spread the disease (Campagnolo *et al*., 2001; Wang *et al*., 2013). Duck plague is caused by Anatid herpes virus 1 belonging to *Herpesviridae* family, *alpha herpesvirinae* sub family, *Mardi virus* genus (International Committee on Taxonomy of Viruses (ICTV), 2014). In India, the disease outbreak recorded from West Bengal (Mukerji *et al*., 1963, 1965 - first report), Karnataka (Bulbule, 1982), Uttar Pradesh (Mukit, 1985), Kerala (Kulkarni *et al*., 1995), Tamil Nadu (Chellapandian *et al*., 2005), and Assam (Konch *et al*., 2009). More than 25 outbreaks were recorded in various districts of Assam state in India during the period from August 2012 to December 2015 (Neher *et al*., 2018).

Clinical signs and pathology included sudden death, partial to complete closure of eyelid, photophobia, polydipsia, loss of appetite, nasal discharge, watery diarrhoea and soiled vent. Grossly, lesions of vascular damage, blood in the body cavities, pseudo membrane in the mucosal secretions, pinpoint haemorrhage and white foci in liver were observed (Santhu and Metwally, 2018). Microscopically, eosinophilic intranuclear and

intracytoplasmic inclusions in the epithelial cells of digestive system were observed.

Duck plague virus can be identified by virus neutralization test (Wu *et al*., 2011; OIE, 2012), passive haemagglutination test (Das *et al*., 2009), inoculation into duck embryo CAM (Hanaa *et al*., 2013) and finally polymerase chain reaction (PCR) (Li *et al*., 2009). The present paper reports on the duck plague outbreak in a Chara-Chemballi duck farm with gross, histopathology and molecular identification in the Tamil Nadu State of India.

Case description

The duck farm had 4500 adult Chara-Chemballi ducks which were recently purchased from Chenganacherry in Kerala State and 2000 birds died in the farm in Kadavakathi village near Tenkasi, Tirunelveli district. Mortality percentage was 44.4.

Necropsy examination was performed. Visceral organs were collected in 10% neutral buffered formalin (NBF). The paraffinised tissue sections were cut into 4-6 µ thickness and stained with haematoxylin and eosin (H&E) stain and examined under light microscope.

Polymerase chain reaction was carried out from homogenised liver tissue. DNA extraction was carried out as per QIAmpKit instructions (Germany). The primers described by OIE (2012) were used for amplification of the targeted DNA sequence. The target gene for PCR amplification is DNA polymerase gene (partial) with a size of 446 bp which is as per OIE terrestrial manual, 2018 and is being used as diagnostic PCR for Duck plague virus. The following primer sequence was used for the detection.

PCR primers for DEV DNA-directed DNA polymerase gene

Primer 1 sequence: 5´ GAA-GGC-GGG-TAT-GTA-ATG-TA 3´ (forward)
Primer 2 sequence: 5´ CAA-GGC-TCT-ATT-CGG-TAA-TG 3´ (reverse)

The template DNA used is 5 pg/µL and master mix (GoTaq Green master mix - 2x, Promega, USA) was used. Thermal conditions used for amplification of the DEV DNA-directed DNA polymerase gene involved initial denaturation for 94°C for 2 min, held at 37°C for 1 min and final holding at 72°C for 3 min. A hold at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min was maintained for 35 cycles and final extension was carried over at 72°C for 7 min and at 4°C until stored.

The amplified PCR product was loaded to the wells of 1% agarose gel for electrophoresis. After that, it was stained with ethidium bromide and was visualised under UV-Transilluminator.

Results

The clinical signs of inappetence, swollen eyes, partial closure of eyelid, conjunctivitis, corneal opacity (Fig. 1), oculonasal discharge, soiled vent with green white watery diarrhoea, ataxia, incoordination and sudden death (Fig. 2) were recorded. The incubation period observed in this outbreak ranged from 3 to 5 days.

Gross pathology involved diphtheritic membrane in the oesophagus (Fig. 3), congestion, petechial haemorrhages and multifocal gray-white areas on the surface of the liver (Fig. 4), epicardial haemorrhages, congested trachea, lung, kidneys and splenomegaly with mottled appearance and enteritis.

Histopathologically, eosinophilic intranuclear and intracytoplasmic inclusions in the epithelial cells of the intestine, degeneration and necrosis of enterocytes, dilated crypt epithelial cells with presence of eosinophilic intranuclear (Fig. 5) and intracytoplasmic inclusions were observed. Enteritis (Fig. 6), multifocal degeneration and necrosis with eosinophilic intranuclear (Fig. 7) and intracytoplasmic inclusions in the hepatocytes, congestion and lymphoid cell depletion in

Fig. 1: Corneal opacity in a Chara-Chemballi duck

Fig. 2: Sudden death in a Chara-Chemballi duck flock

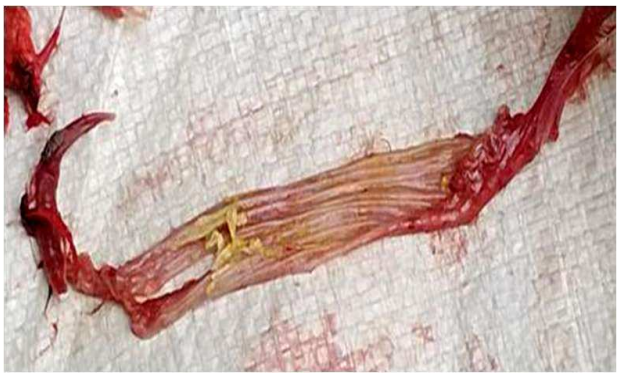

Fig. 3: Chara-Chemballi duck, Oesophagus: Diphtheritic membrane

Fig. 4: Multifocal grey white areas (arrows) on the surface of the liver in a Chara-Chemballi duck

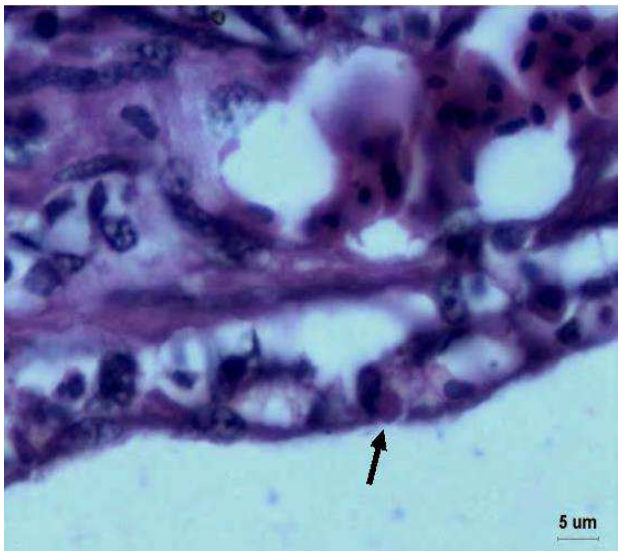

Fig. 5: Presence of eosinophilic intracytoplasmic inclusions in the intestinal epithelial cells (arrow)

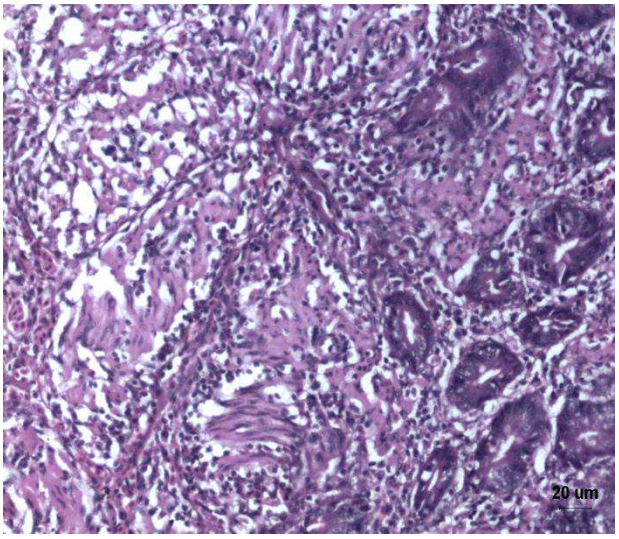

Fig. 6: Congestion, haemorrhages and mononuclear cell infiltration inbetween the crypts

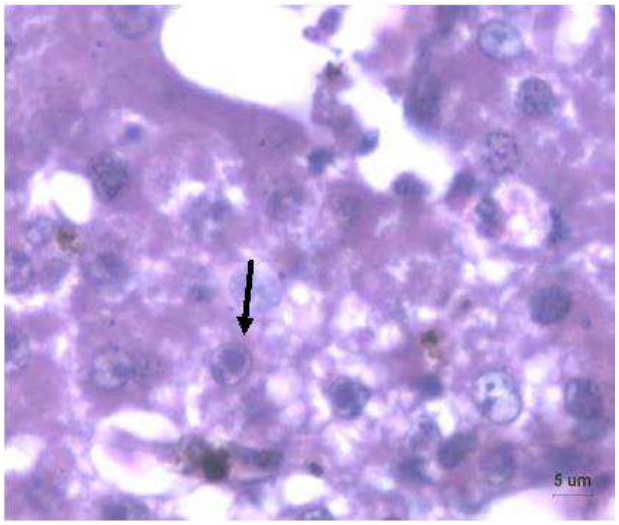

Fig. 7: Presence of eosinophilic intranuclear inclusion (arrow) in the hepatocytes with hepatocellular degeneration

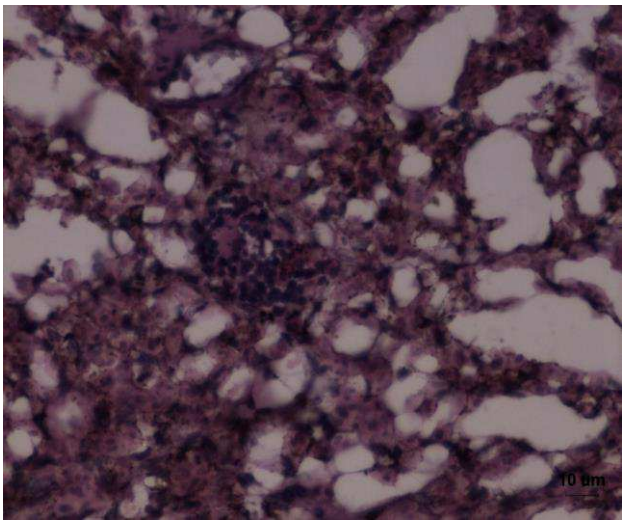

Fig. 8: Mononuclear cell infiltration in the wall of the blood vessels

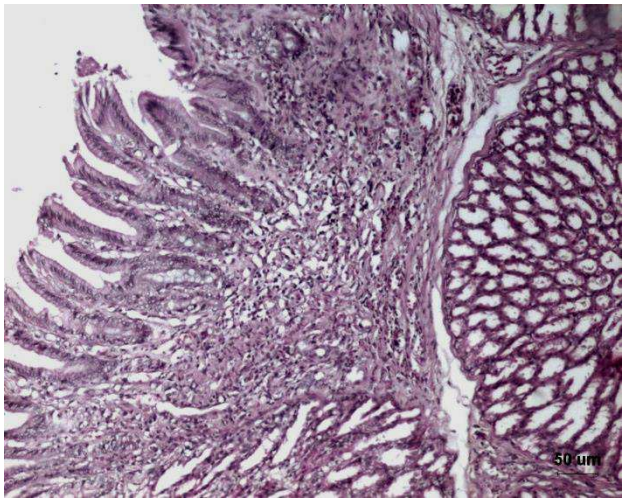

Fig. 9: Mononuclear cell infiltration in the proventricular mucosa

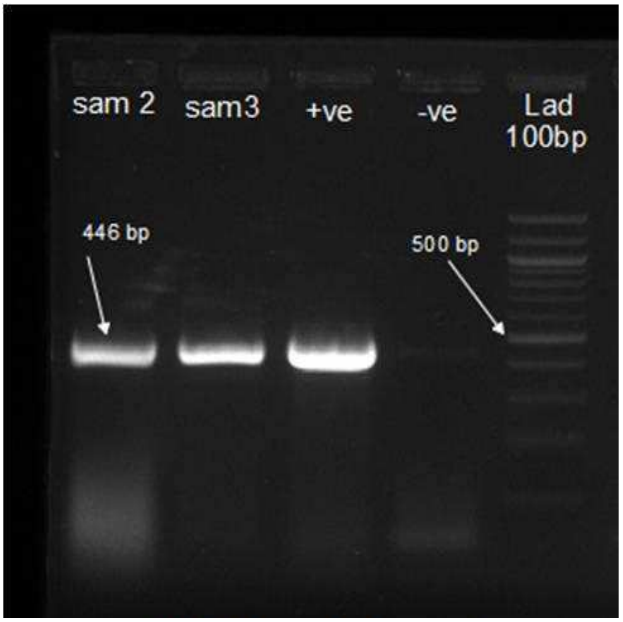

Fig. 10: Amplification of polymerase gene fragment (446 bp) was observed in 1% agarose gel electrophoresis. Ladder: 100 bp, -ve control; +ve control; sample 2 and sample 3 at 446 bp

the spleen, vasculitis (Fig. 8), congestion and haemorrhages in the trachea, lungs, proventriculitis (Fig. 9), congestion and haemorrhages in the kidneys and congestion in the brain were observed.

Amplification of polymerase gene fragment (446 bp) was observed in 1% agarose gel electrophoresis (Fig. 10) which confirmed the etiological agent of duck plague virus infection.

Discussion

Clinical signs recorded in the present outbreak were in accordance with earlier works (Campagnolo *et al*., 2001; Sandhu and Shawky, 2003; Gough, 2008; Sandhu and Metwally, 2008). The mortality percentage was 44.4 in the present case while Sandhu and Shawky (2003) and Carter *et al*. (2006) recorded high mortality of 60 to 90% and 25% drop in egg production. Incubation period was 3-5 days in the present case while Fenner *et al*. (1993) recorded a 3 to 7 day period. The outbreak recorded in March is in agreement with Dhama *et al*. (2017) who reported that 80% of the outbreaks were reported from March to June and National Wildlife Health Center (2011) recorded that the outbreak might be due to stress resulted from hot daylight and onset of breeding that triggers virus release during spring season. Recent purchase of ducks from Kerala through vehicle transport during hot summer season along with heat induced stress might be one of the contributory factors for the infection.

The gross lesions of necrotic changes in the liver, degenerative changes in the lymphoid organs, enteritis, epicardial haemorrhages, congestion in the trachea, lung and kidney, diphtheritic membrane in oesophagus are also recorded by previous works (Campagnolo *et al*., 2001; Sandhu and Shawky, 2003; Sandhu and Metwally, 2008; OIE, 2012). Diphtheritic membrane in the oesophagus was also reported by Campagnolo *et al*. (2001); Sandhu and Shawky (2003), and Konch *et al*. (2009).

In the present case, eosinophilic intranuclear and intracytoplasmic inclusions in the epithelial cells of intestine and liver are in accordance with earlier reports (Shawky, 2000; Campagnolo *et al*., 2001; Konch *et al*., 2009). The present case revealed congestion and haemorrhages in the trachea, lung, spleen, kidney and brain which is in agreement with earlier reports which stated that the congestion and haemorrhages are due to blood vessel leakage (Sandhu and Shawky, 2003; Konch *et al*., 2009). Proventriculitis was recorded in this study while haemorrhages were observed in the proventriculus by Dhama *et al*. (2017).

The amplified PCR product was observed at 446 bp for DNA polymerase gene of DVE which is also recorded by previous investigations (Hansen *et al*., 1999; Wu *et al*., 2011; OIE, 2012; Mostakim Ahamed *et al*., 2015).

In the present study, duck plague viral infection was recorded in the Chara-Chemballi ducks (native breed of Kerala State of India) which occurred due to heat stress during the March month (spring season).

Conflict of interest

Authors declare no conflict of interest.

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