An outbreak of duck hepatitis A virus infection in nomadic ducklings

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Duck hepatitis A virus,
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Summary
During winter of the year 2020, a flock of 9 day-old 5000 non-descript ducklings was affected with huge daily mortality, dullness, depression and opisthotonus. Clinically, there was severe depression, spasmodic paddling and opisthotonus. On post-mortem, liver was enlarged and pale with patchy ecchymoses. Presence of perihepatitis and pericarditis during post-mortem examination of one duckling might be attributed to secondary bacterial infection. Upon completion of disease episode, there was 80 percent mortality in eight days and only less than 20 percent weak ducklings survived. Liver homogenate which was subjected for molecular confirmation through one-step reverse transcriptase polymerase chain reaction (RT-PCR) using primers for RNA dependent RNA polymerase (3D) gene yielded positivity for duck hepatitis A virus (DHAV-1). Histological observation of liver revealed hepatocyte degeneration and necrosis. It is clear that DHAV-1 which is epornitic in nature causes a major devastating disease endangering duck farming.

Introduction

Duck hepatitis, a highly fatal, acute and contagious disease affecting 1 to 3 week-old ducklings with rapid onset and huge mortality (50 to 90%) is caused by duck hepatitis virus which is classified as a sole member of *Avihepatovirus* of *Picornaviridae* family. Duck hepatitis is of paramount significance in duck farming ever since it was first described (Levine & Fabricant 1950). It is also reported that duckling flock of less than 6 week-old may suffer 100% mortality leading to severe economic impact on duck farming (Yugo *et al.* 2016). In India, the report of duck hepatitis caused by virus distinct from DHAV-1 (Rao and Gupta 1967) and presence of duck hepatitis virus B (Sridhar *et al.* 1993) were documented. The disease can be caused by three different viruses namely DHAV type 1, 2 and 3. It is not possible to distinguish among DHAV types on the basis of clinical signs and pathology. The presence of duck hepatitis A virus (DHAV–1) is usually confirmed either by inoculating liver sample on to susceptible duckling, embryonated duck eggs wherein embryos die 24 to 72 hours post-inoculation via allantoic sac route with stunting and haemorrhages or by the inoculation of duck embryo liver cells primary culture of which produce rounding and necrosis of cells (*WOAH* Terrestrial Manual 2018). Different serological tests which are time consuming and labour intensive were useful in diagnosis (Murty & Hanson 1961, Hwang 1969, Zaho *et al.* 1991) and epidemiological study. Although the sudden onset, rapid spread and acute mortality are characteristic of DHAV infection, the virus must be isolated or demonstrated by RT-PCR. Other causes of acute mortality in duckling include salmonellae and aflatoxin both of which do not cause liver lesions suggestive of DHAV infection but cause rapid onset of mortality, ataxia, convulsions and

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opisthotonos in aflatoxicosis (https://www.cabi.org/isc/datasheet/84184). Furthermore, the virus is known to evolve as a new recombinant one through recombination (Rohaim *et al.* 2021).

Alternatively, DHAV RNA may be detected by a one-step reverse transcriptase polymerase chain (RT-PCR) reaction. Further, RT-PCR is the method recommended (+++) for detection of antigen in individual animal, contribution to eradication policies and confirmation of clinical cases of duck virus hepatitis by Wold Organisation for Animal Health (*WOAH* Terrestrial Manual 2018).

Therefore, one-step RT-PCR which is proved to be a rapid and sensitive tool (Kim *et al.* 2007) is applied for diagnosis of DHAV-1 directly from the clinical specimen (Fu *et. al.* 2008) in the present report.

Materials and Methods

Four dead ducklings from a nine day-old non-descript (nomadic type) flock of 5000 were brought for post-mortem to Avian Disease Laboratory, Thalaivasal, south India.

The flock was reported to be affected with 50% mortality in 5 days showing symptoms like severe depression, opisthotonus and spasmodic paddling. Sampling was done from liver (2 numbers) for molecular detection of DHAV-1 and histopathology.

Molecular identification of DHAV-1

RNA isolation: Liver homogenates were subjected for RNA isolation (Qiagen, USA) following manufacturer's instruction.

Primers: For the detection of DHAV-1 nucleic acid, primers for RNA dependent RNA polymerase (3D) gene (Kim *et al.* 2007) ComF- 5'-AAG AAG GAG AAA ATC AAG GAA GG-3') and ComR- 5'-TTG ATG TCA TAG CCC AA G ACA GC-3' flanking a 467 bp DNA sequence in the 3D gene were used.

One-step RT-PCR: Reverse transcription was carried out using Verso cDNA synthesis kit (Thermo Scientific # AB 1453/A). It was performed at 42°C for 30 minutes followed by 95°C for 2 minutes to inactivate the enzyme.

PCR was conducted in a 25 µl reaction mix where Ampliqon red dye master mix 12.5 µl, forward primer (10 pmol/µl) 1µl, reverse primer (10 pmol/µl) 1 µl, nuclease free water 8.5 µl and template DNA 2 µl were added. Reaction was conducted (BioRad C1000 touch) for 35 cycles with following conditions:

denaturation for 45 seconds at 94°C, annealing for 45 seconds at 52°C, elongation for 45 seconds at 72°C and final elongation for 7 minutes at 72°C. Amplified PCR products were run on 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) and the gel was visualized using Gel Doc™ XRT imager with Image Lab™ software.

Histopathology

Liver samples were collected from dead ducklings and fixed in 10% formalin. Sections of 4 µm were cut and mounted on microscope slides that were stained with haemotoxylin and eosin (Bancroft and Gamble 2008).

Result and Discussion

Ailing ducklings exhibited spasmodic paddling and opisthotonus and one of the dead ducklings presented typical posture (Fig. 1).

Figure 1. *Typical opisthotonus in a dead duckling.*

At necropsy, major changes were observed in liver (Fig. 2) which was pale, enlarged with haemorrhagic spots in all the ducklings brought for post-mortem examination.

The above findings were similar to the reports of DHAV–1 infection in ducklings made by other workers (Kozydrun *et al.* 2014, Kamomae *et al.* 2017, Hisham *et al.* 2020). One duckling showed pericarditis and perihepatitis which might be attributed to secondary invasive bacterial infection culture of which revealed *Escherichia coli*. Especially when the immune system of the host is compromised due to other infections like DHAV-1 infection in this case, it is axiomatic that bacteria can easily invade resulting subsequent damage. In other reports, *Salmonella* Enteritidis was isolated from the liver obtained from liver of dead ducklings in DHAV-1 outbreak (Kamomae *et al.* 2017). Additionally, there was haemorrhagic line in exo-occipital region (Fig. 3).

Severe meningial hyperemia and haemorrhage were reported (Niu *et al*. 2019) in dead ducklings experimentally infected with either DHAV- 1 or 3.

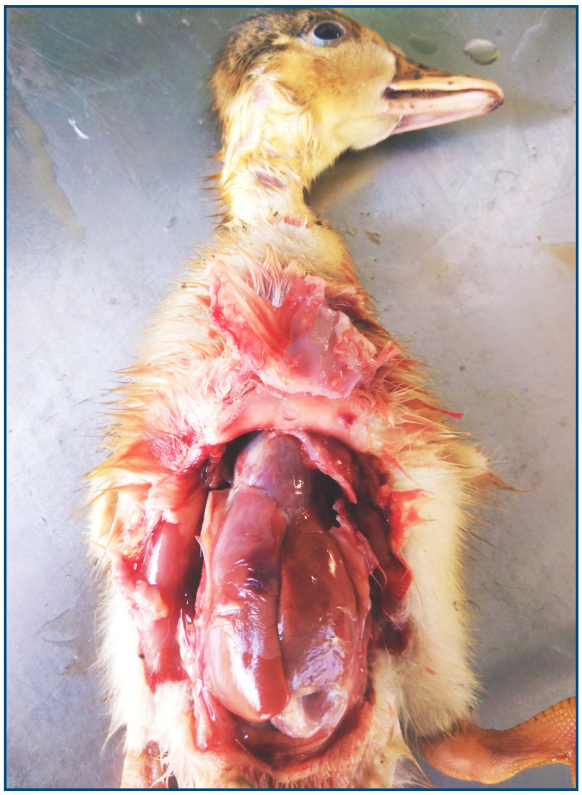

Figure 2. *A dead ducking showing pale liver with ecchymoses.*

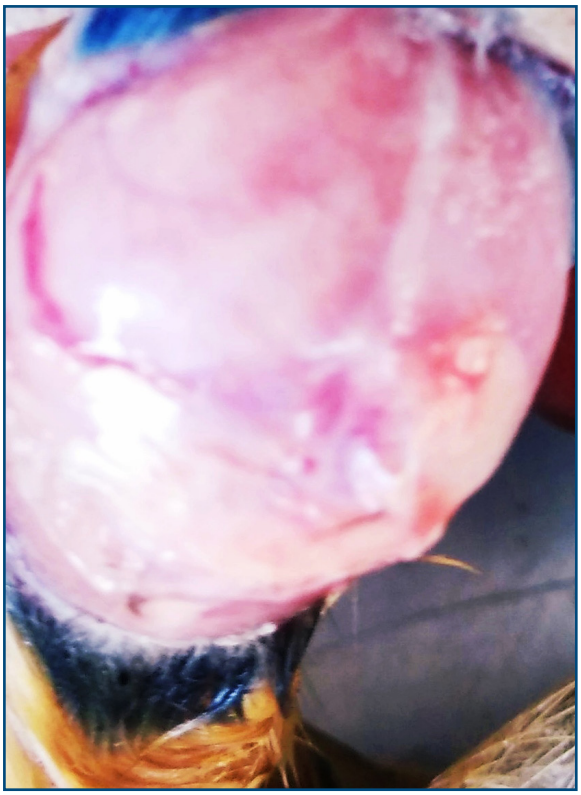

Figure 3. *Haemorraghic line in exo-occipital region.*

Both collected samples were positive for 3D gene of DHAV-1 amplifying 467 bp (Fig. 4) products in one-step RT-PCR.

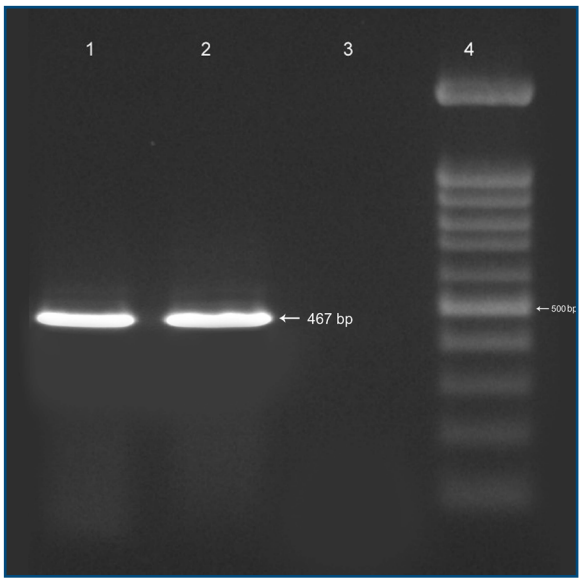

Figure 4. *Electrophoresis of RT-PCR products in 1.5% agarose gel. Lane 1 & 2: Field samples amplifying 467 bp product (3D gene); Lane 3: Negative control; Lane 4: 100 bp ladder.*

It reaffirmed the usefulness of one-step RT-PCR for detecting DHV-1 genome directly from specimens and this observation is in line with the findings of Kim *et al.* 2007, Fu *et al.* 2008, Kozydrun *et al.* 2014 and Kamomae *et al.* 2017. Therefore, application of one-step RT-PCR in diagnosis of DHAV is justifiable as it not only saves time and labour but also facilitates rapid reporting to the concerned authorities. Histologically, there were severe congestion of hepatic blood vessels, diffuse degeneration and necrosis of hepatocytes which is congruous to the findings of Kamomae *et al.* 2017 in natural outbreak and Hisham *et. al.*, 2020 in experimentally infected ducklings. Additionally, proliferation of basophilic ductular cells around the portal areas which is in line with the findings of Kamamae *et al.* 2017 and Niu *et al.* 2019 was also observed (Fig. 5).

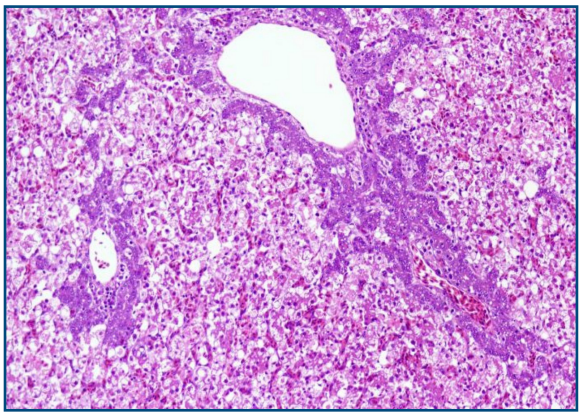

Figure 5. *Histological section of the liver showing diffuse degeneration and necrosis of hepatocytes with proliferation of ductular cells around portal area. Hematoxylin and eosin. 1000x.*

By the end of the epidemic, there was 80% mortality within eight days and the 20% surviving weaklings were disposed taking sufficient care not to spill the infection.

Exposure to DHAV-1 either through contamination in hatchery or through wild bird (Asplin 1961, Kamomae *et al*. 2017) might have led to the disease epidemic.

It is noted that the place of disease occurrence is a non-intensive duck farming area and far from other duck rearing vicinity. Severity of the disease could have been enhanced by stress factors arising due to travel of long distance combined with poor nutrition, as in this case, ducklings were transported for nearly 150 KM on road in search of grazing field.

In India, Rao & Gupta 1967 reported the incidence of duck virus hepatitis which is known to be distinct from DHAV–1 but its relationship to other DHAV types is not known (Kim *et al*. 2008). Sridhar *et al*. 1993 found duck viral hepatitis B antigen to the extent of less than 10% in an organised duck farm of south India.

Current scenario emphasizes the need for molecular epidemiological study of the virus in duck farming area including wild birds to understand the propensity and kinetics of the virus in order to formulate a fruitful strategy for prevention and control of duck virus hepatitis. Prevention of DHAV–1 infection in ducks attains importance as there is possibility of vertical transmission (Zhang *et al*. 2021), apart from epornitic spread of the virus. Although the disease occurred in non-intensive duck rearing area, suitable guidelines were given to animal husbandry department in order to curtail further spread of infection to duck rearing areas.

Conclusion

Molecular identification of DHAV-1 in one week-old duckling flock which suffered 80% mortality within eight days was carried out for the first time in India. It could be concluded that one-step RT-PCR is suitable tool for detection of DHAV-1 genome directly from clinical specimens. It is believed that the role played by wild birds in the spread of DHAV-1 is one of the important factors to be considered while designing the disease preventive strategies. Furthermore, recent occurrence of DHAV-1 infection spells a doom over duck farming.

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