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Inclusion Body Hepatitis in commercial broiler chickens of an organised poultry farm – A case study

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Abstract

Adenoviruses cause a wide range of diseases among all avian species. Even though most of them cause mild disease, some are associated with specific clinical conditions. Fowl adenoviruses (FAdVs) are the etiological agents of two important diseases; Inclusion body hepatitis (IBH) and Hepatitis hydropericardium syndrome (HHS) in poultry. Ten dead birds of 45 days old were brought for necropsy to the post-mortem facility available at the Division of Pathology, ICAR-IVRI, Izatnagar. The percentage of mortality was nearly 15 per cent and affected birds were dull, depressed with pallor of comb and ruffled feathers. On post-mortem examination, it was observed that the liver was swollen and yellow with fibrinous deposits over the surface. On microscopic examination, large basophilic intranuclear inclusion bodies were present in hepatocytes which was suggestive of IBH. Diagnosis was further confirmed by Polymerase Chain Reaction (PCR).

Keywords: *Fowl adenovirus, IBH, hydropericardium, intranuclear inclusion bodies*

Infection with fowl adenoviruses (FAdVs) can cause a range of syndromes in chicken, such as inclusion body hepatitis (IBH) and hepatitis-hydropericardium syndrome (HHS), resulting in substantial economic losses due to mortality and growth retardation in poultry around the world (Schachner *et al.,* 2018). The majority of FAdVs that cause IBH are serotypes FAdV-2, FAdV-11 (species Fowl aviadenovirus D), FAdV-8a and FAdV-8b (species Fowl aviadenovirus E) (Absalon *et al.,* 2017). Helmboldt and Frazier (1963) defined the first case of IBH in chickens as an "acute hepatic catastrophe" due to the severity of liver injury in the affected chickens. In chickens, the disease

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spread both vertically and horizontally, resulting in an array of diseases (Schachner *et al.,* 2018). It mostly affects 3-5-week-old birds, causing severe IBH with mortality ranging from 0 to 17 percent (Niczyporuk *et al.,* 2020). Clinically, afflicted birds exhibit huddling, lethargy, ruffled feathers, and inappetence (Mittal *et al.*, 2014). The main aim of this study was to find out the cause of severe mortality in the organised commercial farm and to study the pathology of the disease in chickens.

A total of ten commercial broiler birds of forty-five days old were subjected for necropsy examination with a history of dull, depression with ruffled feathers. As per owner's statement, the mortality rate of the farm was recorded as 15 per cent. Detailed necropsy was carried out. To study the case further, the liver tissue was taken for cytology, histopathology and PCR. Cytological examination was done by using grease free glass slides. The touch impressions from the cut surface of the liver samples were stained with Field stain. Briefly, 1 cm^3 liver tissues were preserved in 10 per cent neutral buffered formalin. The formalin preserved tissue samples were studied histologically using Haematoxylin and Eosin staining (Suvarna *et al.,* 2019). The tissue sample was also preserved in -20°C for molecular screening by PCR. DNA was extracted from liver tissues by DNASure Tissue Mini Kit (Genetix) as per the manufacturer's instruction. The confirmative diagnosis was done using PCR by targeting Hexon gene of Fowl adenoviruses (FAdV). The gene was amplified using a conventional PCR. The primer pair FP: 5'-CAARTTCAGRCAGACGGT-3' and RP: 5'-TAGTGATGMCGSGACATCAT-3' was used as per the previous study by Ottiger (2010) and Meulemans *et al*. (2001). The PCR products were visualized by agarose gel electrophoresis.

The percentage of mortality in the farm was 15 which is similar to the previous studies where the mortality rate for broiler chicks was 20% in the 30- to 40-day age range, whereas the mortality rate for broiler breeder chickens was 2.5% at the 7-8-week age range (Philippe *et al.,* 2005). The greater incidence in commercial broiler chickens may be associated to the stress of achieving maximal development and the waning of maternal antibodies at that time. The external examination on the dead birds during necropsy revealed pallor of comb and wattles, ruffled feathers. Sudden mortality, varied degrees of dullness, reduced feed intake, ruffled feathers, watery diarrhoea; changes in posture, and difficulty walking were all common the clinical signs observed in infected flocks (Schachner *et al*., 2018). The internal organs including heart, lungs, liver and kidneys were examined for gross lesions. The contour of the liver from all the birds examined were swollen, mottled, friable in consistency, mild to moderate yellowish in colour and the edges were round (Fig. 1). The gross pathology of liver coincides with the work of Zhao *et al.* (2015) with similar gross lesions observed in liver with necrotic foci associated with IBH. Kidneys were found to be enlarged, severely congested, with a prominent tubular pattern (Fig. 2). The gross appearance of the liver, in particular with colour and contour, helps to elucidate few differentials such as fatty liver and Inclusion body hepatitis etc. The observations were similar to the findings of

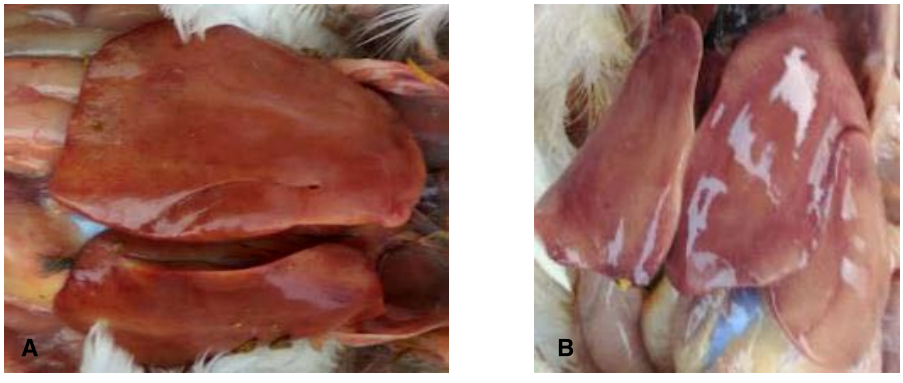

Fig. 1. Swollen, yellow, friable and mottled liver with petechiae

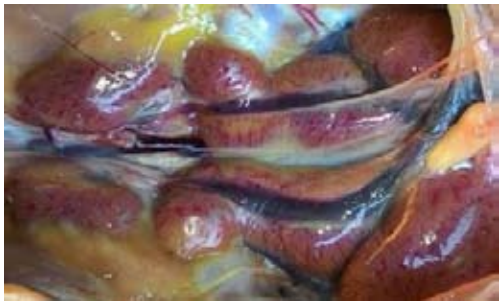

Fig. 2. Kidneys were enlarged and severely congested

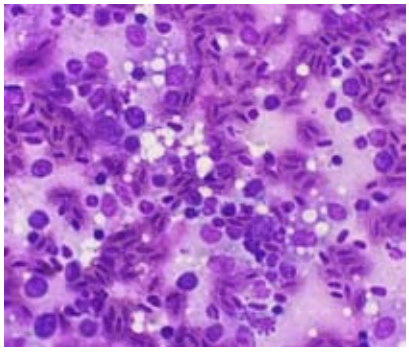

Fig. 3. Impression smear of liver showing fat vacuoles within the hepatocytes (Field stain, 20X)

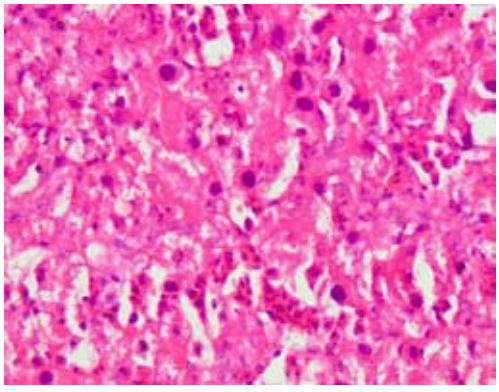

Fig.4. Liver: karyorrhexis and karyolysis of hepatocytes with large intranuclear basophilic inclusion bodies (arrows) (H &E, 20X)

Balachandran *et al.* (1993), Kumar *et al.* (2013) and Sharma *et al.* (2014). In most of the studies, the gross findings in IBH positive cases revealed hydropericardium which had straw coloured fluid in pericardial sac (Balachandran *et al.*, 1993). In the present study, hydropericardium was not an evident finding which was similar to the work of Rahimi and Haghighi (2015).

Fig.5. FADV specific amplicons visualised by agarose gel electrophoresis

Lane M: Molecular weight marker

Lane 1: positive control

Lane 2,3: Field samples showing FADV-specific 897 base pairs product (bp)

Lane 4: negative control

The cytological examination revealed numerous vacuoles in hepatocytes with changes in nuclear structures such as condensation of chromatin (Fig. 3). The observations were similar to those made by Miller *et al.* (2013). Histological examination of the liver revealed degeneration of hepatocytes with vacuolation in the cytoplasm. The nuclei of the liver also showed karyorrhexis and karyolysis with presence of large basophilic intranuclear inclusion bodies (Fig. 4) which was suggestive of IBH. The results from the present study corroborated with the findings of Khodakaram-tafti *et al.* (2016) and Abghour *et al.* (2019) wherein, large viral inclusions in nuclei of hepatocytes were found in Fowl adenovirus (FAdv) affected chicken. Vacuolar degeneration, significant hepatic cord disruption, sinusoidal dilatation and haemorrhages, and basophilic intranuclear inclusions are the common histopathological findings (Balachandran *et al.,* 1993). Similar findings were observed in this study. For the identification of FAdV, polymerase chain reaction is an effective and sensitive technology as opposed to other traditional diagnostic techniques (Ganesh *et al.,* 2002). A region of 897-bp in hexon gene L1 variable using primer pair hexon A and hexon B (Meulemans

et al., 2001) was carried out. The primary surface protein of adenoviruses, known as the hexon protein, contains antigenic determinants that are type-, group-, and subgroup-specific (Russell, 2009). Liver samples were positive for FAdV with amplicon size of 897bp (Fig. 5) and thus the cases are confirmed as IBH. It is recommended that more research be done to identify the IBH serotype in each epidemic in order to develop effective vaccines and stop the spread of infection.

Conclusion

This study confirms the FAdv in broiler chicken by histopathological and molecular investigations. PCR was found to be a highly specific diagnostic tool for confirmative diagnosis of FAdV. Regarding control measures, prophylactic vaccination of entire flock and implementation of a robust biosecurity policy to avoid environment contamination and seems to be the only way to avoid this disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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