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Hexon gene based molecular detection of fowl adenovirus associated inclusion body hepatitis in commercial broiler chicken

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Abstract
Inclusion body hepatitis (IBH), caused by fowl adenoviruses, is an economically important poultry disease that causes substantial economic losses to the world poultry industry. The avian adenovirus was first isolated from an outbreak of respiratory disease in quail (Olson, 1950). Aviadenoviruses affect birds, particularly chickens, ducks, geese, turkeys and pheasants. There are total 12 serotypes of Fowl adenovirus viz. FAdV-1, FAdV-2 FAdV-3, FAdV-4, FAdV-5, FAdV-6, FAdV-7, FAdV-8a, FAdV-8b, FAdV-9, FAdV-10 and FAdV11 which were classified on the basis of restriction fragment length polymorphism (RFLP) profile and sequencing data (Benko*et al*., 2000). FAdV-11 is one of the primary causative agents for IBH and the disease has been reported in many countries worldwide. Recently, inclusion body hepatitis outbreaks have been increasingly reported, particularly in broiler flocks, in different regions of India, i.e., Uttar Pradesh, Madhya-Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Odisha, West Bengal, Chhattisgarh and Mizoram (Gupta *et al*., 2007, Asthana *et al*., 2013, Suohu and Rajkhowa, 2021, Chitradevi *et al*., 2021). On 01/12/2024, in a well-organized poultry farm in Jabalpur region of Madhya-Pradesh, mortality was reported in a 36-day old broiler flock with capacity of 5400 birds in house. Visited Farm and based on the symptoms and postmortem examination of dead birds, inclusion body hepatitis was suspected in affected flock. Samples were collected for further diagnosis. On microscopic examination, large basophilic intranuclear inclusion bodies were present in hepatocytes which was suggestive of IBH. Polymerase Chain Reaction (PCR) was done using 897 bp hexon gene for confirmative diagnosis.

Keywords: Fowl adenovirus, IBH, intranuclear inclusion bodies, PCR

Introduction
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) [14]. The majority of FAdVs that cause IBH are serotypes FAdV-2, FAdV-11 (FAdV D), FAdV-8a and FAdV-8b (FadVE) (Absalon *et al*., 2017) [1]. 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 spread both vertically and horizontally, resulting in an array of diseases (Schachner *et al*., 2018) [14]. It primarily infects broilers of 1 to 5 weeks of age and occasionally layer and breeder pullets aged 10 to 20 weeks. In broilers it causes sudden increase in mortality from slight increase to over 30% (Dhahiya *et al*., 2002) [5] and may reach up to 80% in presence of immunosuppressive factors (Kumar *et al*., 2003) [8], whereas the mortality rate for broiler breeder chickens was 2.5% at the 7-8-week age range (Philippe *et al*., 2005) [12].

Postmortem examination and sample collection
Affected birds at the farm displayed symptoms such as ruffled feathers, red-tinged faeces including undigested feed, nasal discharge, head swelling, lameness in a some of birds, dullness, depression, and odd respiratory sounds. On post-mortem examination of 15 birds, it was observed that liver was friable, pale, enlarged with haemmorrhagic spots over the

surface, spleen enlarged, kidneys were congested with white urate deposits in ureters, pericardial fluid was present in excess with Leechi shaped heart, proventriculus was enlarged with haemmorrhagic spots inside, misshapen gizzard and enteritis (Fig.1 and 2). There was approximately 15% mortality which continued for 15 days. Liver tissue samples were collected for histopathology and PCR from ten dead birds. 1 cm^3 liver tissues were preserved in 10% neutral buffered formalin which were studied histologically using Haematoxylin and Eosin staining (Suvarna *et al*., 2019) [16]. The tissue samples were also preserved at-80 °C for molecular screening by Polymerase Chain Reaction (PCR).

DNA isolation and polymerase chain reaction

DNA was extracted from liver tissues by standard phenol-chloroform method. Quality and quantity check of extracted DNA was done using Nanodrop system (Fig.3). The confirmative diagnosis was done using PCR by targeting hexon gene of Fowl adenoviruses (FAdV). The primer pair FP: 5'-CAARTTCAGRCAGACGGT-3' and RP: 5'-TAGTGATGMCGSGACATCAT-3' was used as per the previous study by Ottiger (2010) [11] and Meulemans *et al*. (2001) [9].

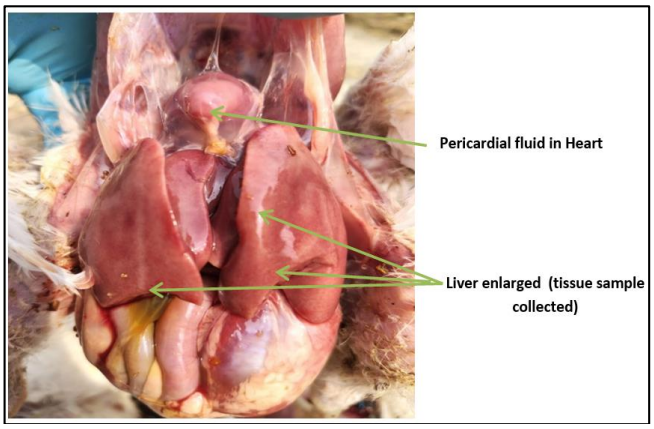

Fig 1: Liver enlarged, pale and heart having pericardial fluid

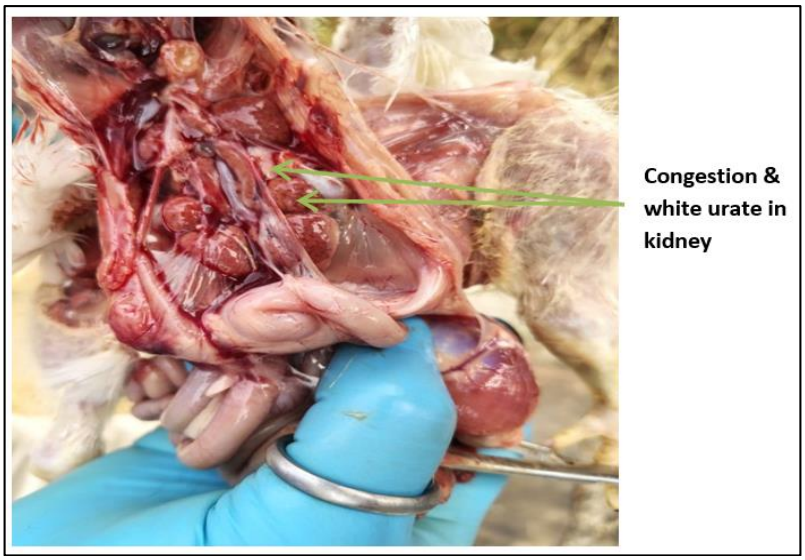

Fig 2: Kidneys congested and having white urate deposits

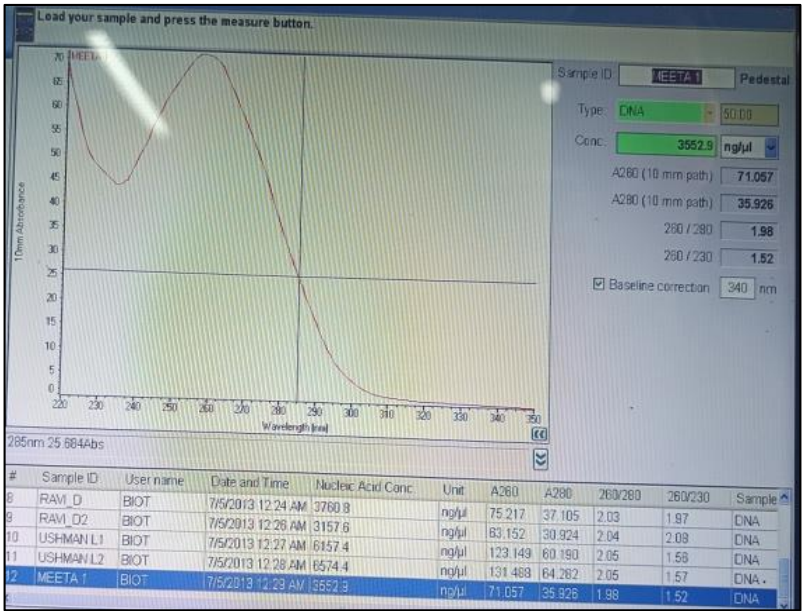

Fig 3: Quantification of DNA by Nanodrop Spectrophotometer

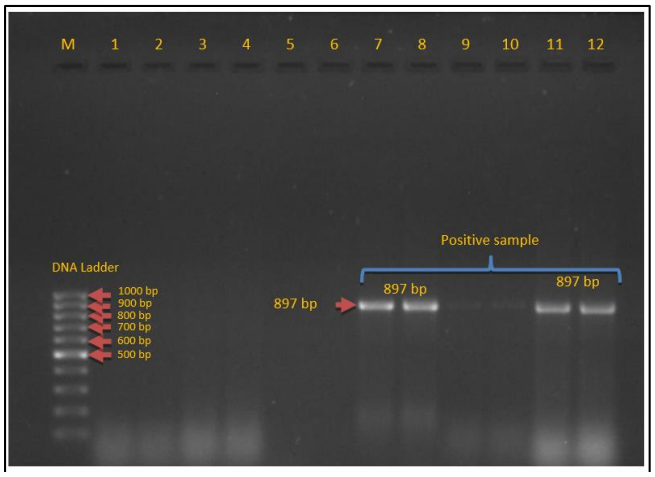

Fig 4: PCR product visualized on 1.2% agarose gel electrophoresis confirmed positive for FAdV with amplicon size 897

Amplification of 897 bp in hexon gene L1 variable region was done by primer pair hexon A and hexon B using suitable PCR reaction mixture and protocol with Ta 56 °C. Hexon protein is the primary surface protein of adenoviruses which contains antigenic determinants that are type, group and subgroup specific (Russell, 2009) [13]. The PCR products were visualized by 1.2% agarose gel electrophoresis in Geldoc system (Fig.4). Liver samples were positive for FAdV with amplicon size of 897 bp and thus the cases are confirmed as IBH.

Results

In the present study, inclusion body disease (IBH) was suspected on the basis of symptoms and postmortem examination in a commercial broiler flock of 5400 capacity in Jabalpur, Madhya-Pradesh, India. IBH was confirmed by histopathology and Hexon gene specific polymerase chain reaction. Basophilic intranuclear inclusion bodies were found in hepatocytes on histopathological examination and 897 bp amplicon fragment was seen in agarose gel electrophoresis, which was suggestive of FAdV associated IBH infection.

Conclusion

This study confirms the IBH in broiler chicken at a poultry farm by histopathological and molecular diagnosis. PCR was found to be a highly specific diagnostic tool for confirmative diagnosis of the disease, as IBH is misdiagnosed with other viral diseases and toxicity. IBH alone can cause higher mortality in affected poultry flock, but when mixed infected with other immunosuppressive factors, mortality will increase to a higher rate. Prophylactic vaccination and strict biosecurity measures should be followed to keep the disease away from the farm. It is recommended that more research be done to identify the IBH serotype in each outbreak to develop effective vaccines and stop the spread of infection.

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Conflict of interest

The authors declare that they have no conflict of interest.

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