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SHORT COMMUNICATION

Pathology and molecular characterization of Inclusion Body Hepatitis-Hydropericardium Syndrome complicated with coccidiosis in broilers: A report

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ABSTRACT

Inclusion body hepatitis (IBH) is a viral disease caused by Fowl adenovirus (FAdV) affecting poultry mainly, broilers. IBH has a worldwide distribution and is endemic in many states of India. Present investigation was carried out on a flock of 27 days old commercial broiler chickens from the Nagpur region with history of yellow mucoid to red diarrhea and an overall mortality of 25%. Grossly, liver revealed pale colour, friable with pinpoint to ecchymotic haemorrhages and necrotic foci. Microscopically, the liver revealed hepatic degeneration with intranuclear dense basophilic inclusion bodies, vacuolation, and rounding of hepatocytes and hemorrhages. The collected samples were screened for the hexon gene of fowl adenovirus through PCR. Phylogenetic analysis revealed that the current FAdV is closely related to the strain isolated from Pantnagar, India.

Keywords: Broiler, coccidiosis, IBH, molecular characterization, pathology, PCR

Fowl adenovirus is known to cause important diseases in poultry birds such as inclusion body hepatitis, hepatitis hydropericardium syndrome, and gizzard erosion and ulceration[1]. Among these conditions, Inclusion body hepatitis has a worldwide distribution and is endemic in many states of India[2]. FAdV has 5 species A to E and 12 serotypes[3]. All 12 serotypes of the group-I FAdVs have been incriminated in the field outbreaks of IBH-HPS, however, FAdV serotype 4 (FAdV-4) had been mostly implicated. Inclusion body hepatitis is characterized by sudden onset of mortality, severe anemia and enlarged, pale, friable fatty liver with hemorrhages with basophilic or eosinophilic intranuclear inclusion bodies in hepatocytes. In recent years, IBH-HPS has become one of the more common impediments with mortality ranging from 10-30% by virulent strains[4]. Coccidiosis, on the other hand, is associated with poor management practices and results in economic losses due to hemorrhagic enteritis. This case report describes the pathology and molecular characterization of IBH-HPS complicated with coccidiosis in broilers.

Ten numbers of 27 days old commercial broiler chickens from the Nagpur region were brought for post-mortem examination to the Department of Veterinary Pathology, Nagpur Veterinary College, Nagpur during March 2023. The flock had a history of yellow mucoid to red diarrhea and an overall mortality of 25%. A detailed post-mortem examination was conducted. Tissues comprising the liver, kidney, heart, intestine, pancreas, spleen and bursa were collected for histopathological examination in 10% neutral buffered formalin. The samples of liver were stored at -20^{O}C for molecular detection of Fowl Adenovirus-I. After fixation, these tissues were processed using xylene and alcohol followed by impregnation in paraffin wax (Qualigens) as per routine method and 5µ sections were cut and stained with H&E stain for recording histopathological observations under light microscopy (Nikon)[5]. The genomic DNA from the liver was isolated using Hi-media DNA purification kit as per manufacturer's protocol. The hexon gene was amplified using published forward primer F-CAARTTCAGRCAGACGT and reverse primer R-TAGTGATGCGSGACATCAT[6]. PCR reaction of 20 µl containing 10 µl of 2X master mix (Promega, USA), 1µl each of forward and reverse primers (10 pmol), 3µl of DNA, and 5µl of Nuclease free water was set for amplification of hexon gene. PCR was performed in an automated thermal cycler (Hi-media) under following conditions: initial denaturation at 95^{O}C for 5 min, followed by 30 cycles of denaturation at 94^{O}C for 45 sec, annealing at 60^{O}C for 1 min and extension at 72^{O}C for 1 min. Final extension was conducted at 72^{O}C for 10 min. A volume of 10 µl of the PCR product was separated in 1% agarose gel

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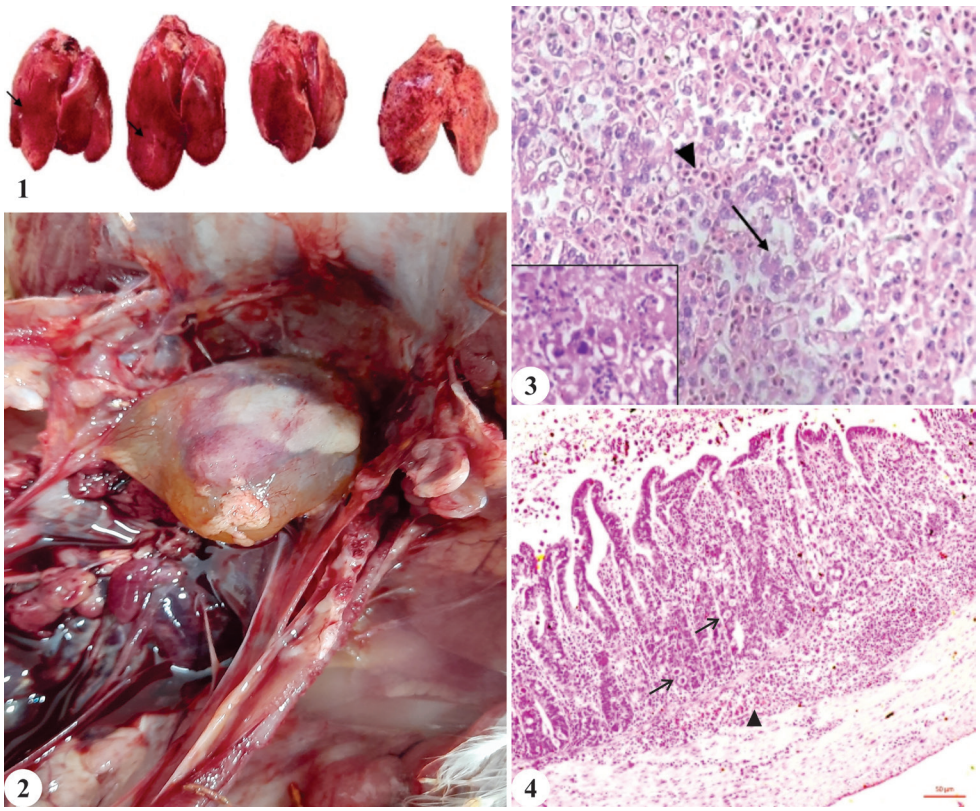

Fig. 1. Enlarged pale liver with haemorrhages and necrotic foci; **Fig. 2.** Hydropericardium; **Fig. 3.** Basophilic inclusion bodies inside hepatocytes (inset) and hepatic degeneration (H&E x200); **Fig. 4.** Sporocysts of coccidia in intestinal mucosa (H&E x200).

by electrophoresis. PCR amplicons of the hexon gene segments of the pathogen was sequenced commercially. The gene segments were trimmed and contig sequences were prepared in Bioedit software. These contig sequences were aligned and the phylogenetic tree was constructed using the Neighbor-joining method using Kimura 2 parameter model using MEGA 7 software[7]. The sequence was submitted in GenBank having accession number OR858637.

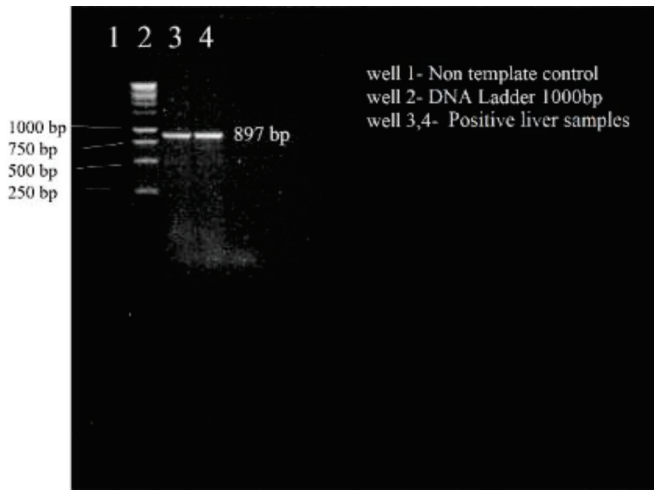

Fig. 5. Gel electrophoresis of hexon gene of fowl adenovirus. Lane 1: Non Template Control; Lane 2: Ladder; Lane 3 & 4: Sample.

Grossly, on post mortem, liver revealed pale colour, friable with pinpoint to ecchymotic haemorrhages and necrotic foci (Fig. 1). The heart revealed hydropericardium, and erosions of mucosa in gizzard, congested, enlarged, and mottled kidneys and haemorrhagic typhlitis was also observed (Fig. 2). Moreover, the spleens were slightly enlarged and had necrotic foci and the pancreas was swollen and had hemorrhagic spots.

Microscopically, the liver revealed hepatic degeneration with intranuclear dense basophilic inclusion bodies, vacuolation and rounding of hepatocytes and hemorrhages (Fig. 3). Degenerative changes along with lymphocytic infiltration in cardiac muscle were observed. Kidneys revealed tubular necrosis along with hemorrhages. Loss of acinar arrangement and shrinkage of acinar cells was evident in the pancreas. Caecum revealed numerous developmental stages of *Eimeria spp*. in mucosa along with hemorrhages in muscularies layer (Fig. 4). Microscopic examination of intestinal scrapping revealed oocysts of *coccidia spp.* in a wet smear at 200x magnification.

The primer specific for hexon gene of fowl adenovirus yielded amplicons of 897 bp (Fig. 5). The BLAST hit result of the nucleotide sequence revealed the closest identity of the isolate with IBH previously isolated in India (Fowl adenovirus D isolate Pantnagar/H-15/R-37/Hexon protein

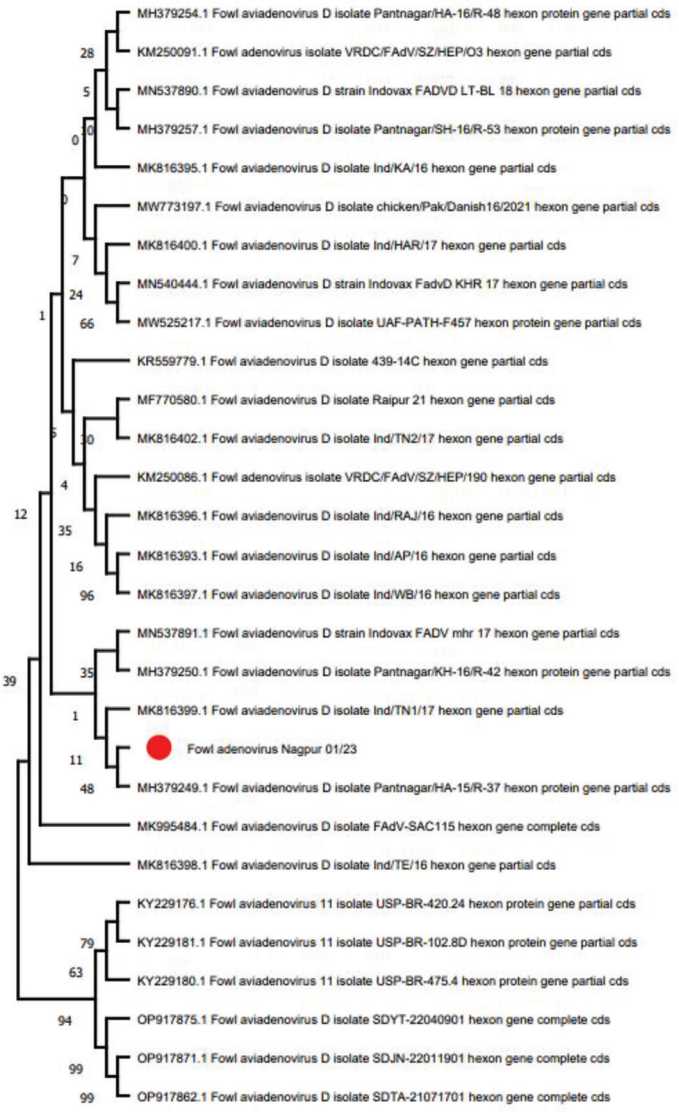

Fig. 6. Phylogenetic tree of nucleotide sequences of the hexon gene of PCR fragments of fowl adenovirus.

gene; MH379249) (Fig. 6).

IBH outbreaks caused by different FAdV serotypes have been described in different countries including India in recent years[8]. IBH can affect broilers of all ages, young chicks are found to be more susceptible during the first two weeks. There is a clear age effect with avian adenoviruses, as the age of the host increases, the degree of multiplication of the viruses within the host is restricted and the mortality decreases[9]. In this study, mortality of about 25% started at 24th day of age and peaked at 27th day in affected broilers. Mortality during IBH outbreaks is normally between 2% and 10% of the flock, but up to 30% has been described in case of co-infection with other immunosuppressive agents[10]. In the present study, coccidiosis was also evident which can easily occur in birds with concurrent disease further worsening the economics of farms.

The gross lesions observed in this study like pale, friable liver with pinpoint to ecchymotic haemorrhages and necrotic fociare characteristic of adenoviral infection. Hydropericardium, and erosions of mucosa in gizzard, congested, enlarged, and mottled kidneys were described by many researchers before and correlated with affection of the liver primarily, which results in decreased liver function and thus decreased protein synthesis leading to hydropericardium[11,12].

The reported disease outbreak in broiler birds was diagnosed as inclusion body hepatitis-hydropericardium syndrome complicated with coccidiosis. The strain, causing the disease was phylogenetically closely related to the Indian strain from Pantnagar. More data is required to know the molecular epidemiology of the fowl adenovirus in central India. Understanding the genetic background of circulating fowl adenoviral strains in India will help to formulate control strategies using vaccines and/or the use of therapeutics. These results indicated that preventive measures against FAdV infection on poultry farms should be implemented.

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