

Research Article

Investigation of Hepatitis–Hydropericardium Syndrome Caused by Fowl Adenovirus (FAdV) Infection in Broiler Chicken Flocks in Northern Parts of India

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ABSTRACT

The present study describes disease investigation of outbreaks of hepatitis–hydropericardium syndrome (HPS) in three different commercial broiler flocks each with a flock size of 5000, 10000, and 20000 birds respectively, of different age groups ranging from 3 to 5 weeks in western Uttar Pradesh and Srinagar region of India. Representative broiler birds (n= 15) from these flocks were brought for postmortem investigation and diagnosis. History obtained from the flock owners revealed a large number of clinically sick birds and varying mortality rates (10–15% (10% in one farm and 15% in two farms). In all the three flock in spite of routine vaccination against major viral diseases (Marek’s disease and infectious bursal diseases). Necropsy examination of the dead and sacrificed birds showed enlarged, pale, friable liver; swollen kidneys with alternating areas of pale and hemorrhagic parenchyma; and varying degrees of hydropericardium. Representative tissue samples of liver, spleen and kidney were collected for histopathology and molecular detection of the HPS causative virus. Histopathologically, there were varying degrees of hepatitis, nephrosis to nephritis, hemorrhages in hepatic and renal parenchyma. Moderate number of hepatocytes showed the presence of large basophilic intranuclear inclusion bodies. Polymerase chain reaction (PCR) testing of the clinical tissue samples, using *Hexon* protein gene specific primers, yielded virus specific amplicon of 897 bp size confirming the presence of fowl adenovirus (FAdV). The recurrent outbreaks of infectious hepatitis – hydropericardium syndrome in broiler industry in different parts of India need to be investigated further to assess the economic losses due to this disease as well as to adapt suitable prevention and control measures.

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INTRODUCTION

Hepatitis–hydropericardium syndrome is one of the important pathological manifestations most commonly associated with adenoviral infection in birds (Balamurugan and Kataria, 2004; Kataria et al., 2013). Adenoviral infections are frequently reported among birds including domestic poultry and wild species of birds but majority of these viruses do not produce apparent clinical signs as a primary etiological agent. However, a few adenoviruses are directly associated with disease conditions and symptoms such as hydropericardium syndrome (HPS), inclusion body hepatitis (IBH), gizzard erosions, respiratory illness, reduced egg production, enteritis, reduced feed conversion, and retarded growth depending upon the serotypes or genotypes involved (Rahul et al., 2005; Domanska–Blicharz et al., 2011; Asthana et al., 2013). The adenoviruses that is associated with these afore mentioned lesions are grouped under the genus *Aviadenovirus*. The aviadenovirus is further subdivided into five distinct genotypes (A–E) with 12 different serotypes.

These viruses are shed in all secretions and excretions, particularly in the feces, which act as the main source of infection to other healthy birds of a flock (Benjamin et al., 2013). The fowl adenoviruses (FAdVs) are diagnosed routinely by isolation of the virus in embryonated chicken eggs or in cell culture, demonstration of virus particles by electron microscopy, or by detection of viral genome by polymerase chain reaction (PCR) (Lim et al., 2012). The virus has affected poultry industry worldwide (Asthana et al., 2013; Kataria et al., 2013) and in past two decades and during recent years many disease outbreaks have been reported from different parts of India (Dahiya et al., 2002; Memon et al., 2006; Gowthaman et al., 2012).

The present paper describes the disease outbreaks of hepatitis–hydropericardium syndrome associated with aviadenovirus infection in broiler chicken flocks in northern states of India and the investigations carried out based on clinical, post–mortem, histopathological examination of affected poultry birds and the molecular detection of the

causative virus. The study adds to the epidemiological data available with regard to this virus, seeing other recent reports of disease outbreaks of FAdVs/HPS in the country appropriate prevention and control measures are suggested to alleviate the economic losses to the poultry producers from this economically important virus/disease.

MATERIALS AND METHODS

Source of Samples

Representative clinical tissue samples from liver, spleen and kidneys of each dead and sacrificed birds (n=5) of the affected farms collected during outbreaks of hepatitis-hydropericardium syndrome (HPS) in three different commercial broiler flocks of different age groups (3 to 5 weeks) in western Uttar Pradesh (2 farms) and Srinagar region (1 farm) of India, have constituted as the study materials for disease investigation based on histopathological examination and polymerase chain reaction (PCR) testing for the presence of fowl adenovirus (FAdV).

Histopathology

Tissue samples of 2–3 mm thickness from liver, spleen and kidneys were fixed in 10% neutral buffered formalin and processed for histological examination as per routine paraffin embedding technique (Luna et al., 1968). Tissue sections of 4–5 μ thickness were cut and stained with Hematoxylin and Eosin for histo-pathological evaluation.

PCR Testing for Detection of FAV

Viral DNA was extracted from the infected liver, spleen and kidney samples using DNeasy Tissue Kit (Qiagen, Vienna,

Austria) as per the manufacturer's protocol. For conventional PCR, 1 μ l of DNA was amplified using 10 pmol of each primer (Forward, 5'- CAA RTT CAG RCA GAC GGT -3'; Reverse, 5'- TAG TGA TGM CGS GAC ATC AT - 3'). The gene encoding LI region of the hexon protein of fowl adenovirus group-I was chosen to select primers (Thakor et al., 2012). The reaction mix was amplified in thermocycler (Quanta Biotech, United Kingdom) by initial denaturation at 95 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 30 seconds; annealing at 57 °C for 30 seconds; elongation at 72 °C for 1 minute followed by final elongation at 72 °C for 4 minutes. The PCR amplified products were separated by electrophoresis in a submerged 1% agarose gel and visualized under ultraviolet light in a Gel Documentation System.

RESULTS

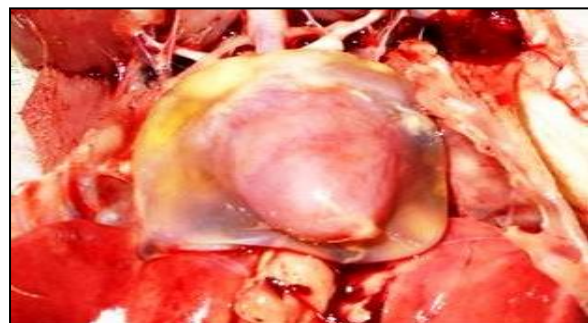
History, Clinical Signs and Gross Pathology

History from the flock owners revealed that the flock size varied from 5,000 to 20,000 birds and the mortality was relatively high in birds aged between 3–5 weeks than in the other age groups in spite of good management practices.

The clinical signs in the affected broiler birds consisted of depression, reduced feed and water intake, reluctance to move, ruffled feathers, huddling, prostration followed by death (10–15%). The necropsy lesions included, pale, friable liver with areas of congestion; varying degrees of hydropericardium containing clear, straw colored fluid; and swollen kidneys with alternating areas of pale and hemorrhagic parenchyma; the bursa of Fabricius was enlarged and edematous (Figure 1).



A



B



C



D

Figure 1: A. Enlarged, pale, friable liver with areas of congestion; B. Pericardial sac containing clear transudate; C. Swollen kidneys with alternating areas of pale and hemorrhagic parenchyma; D. Enlarged and edematous bursa of Fabricius

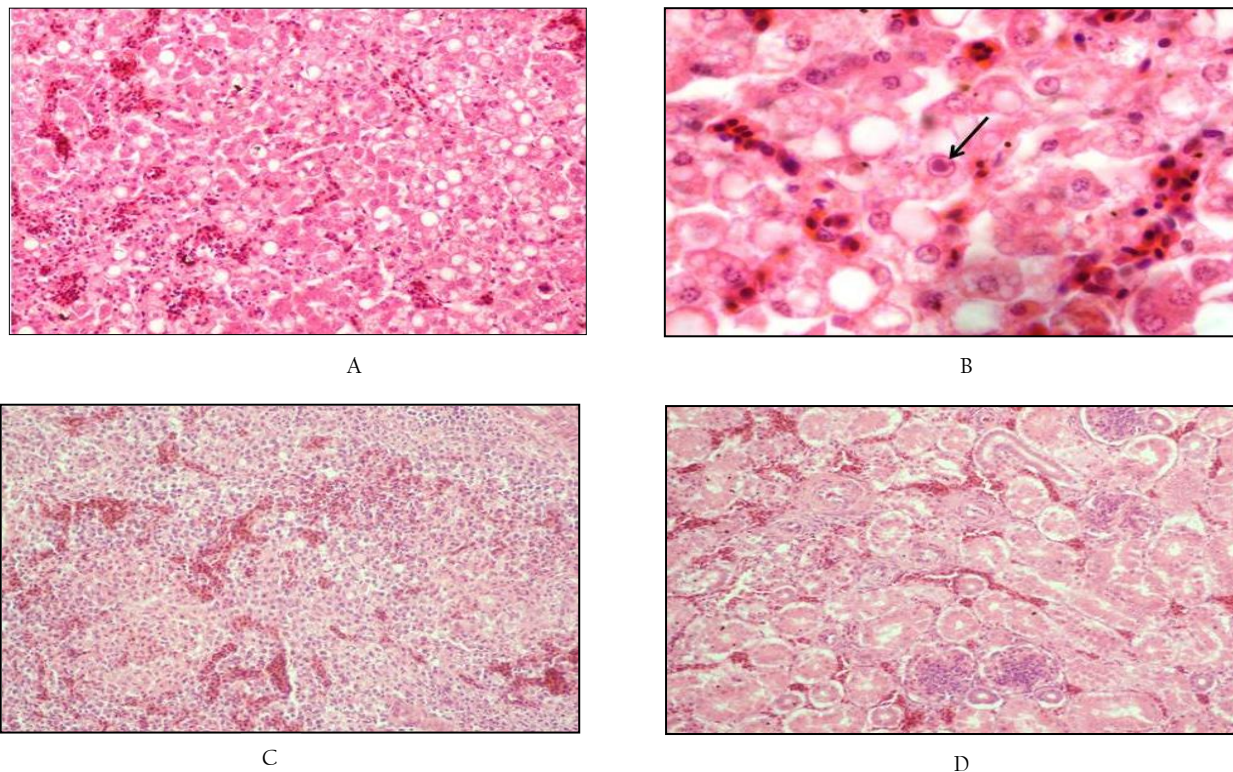


Figure 2: A. Section of liver showing sinusoidal congestion, severe fatty changes and necrosis of hepatocytes amidst mononuclear infiltration; B. Higher magnification of Figure 2: A showing large basophilic intranuclear inclusion body in a degenerating hepatocyte (arrow); C. Section of spleen showing congestion, hemorrhages and lymphoid degeneration; D. Section of kidney showing severe interstitial hemorrhages and degenerative to necrotic changes of renal tubules.

Histopathology

Histopathological findings encountered in the liver were congestion, areas of focal hemorrhages and hepatitis. The hepatic cords in majority of the cases were dissociated and moderate number of hepatocytes showed varying degrees of fatty changes and necrotic changes (Figure 1A) amidst focal collections of leucocytes predominated by mononuclear cells. In a few hepatocytes, the nuclei had disappeared completely, leaving a ghost cell consisting of one or more large vacuoles. Varying degrees of pyknosis, karyorrhexis and karyolysis were evident in the majority of the hepatic cells. In some hepatocytes, karyomegaly and margination of the chromatin was apparent. A few number of the degenerating hepatocytes showed large basophilic intranuclear inclusion bodies which were surrounded by a clear halo with an irregular in outline (Figure 2B). Sections of spleen were characterized by sub-capsular hemorrhages and congestion amidst hemorrhages of parenchyma (Figure 2C). Kidneys showed severe congestion and interstitial hemorrhages along with degenerative and necrotic changes in tubular epithelia (Figure 2D). Bursa of Fabricius from few infected birds revealed inter-follicular edema and degenerative changes.

PCR Detection of FAV in Clinical Samples

The PCR products visualized in agarose gel electrophoresis showed the presence of amplified DNA products of 897 bp in all the amplified DNA samples from tested tissues (liver,

spleen and kidney), confirming the presence of *hexon* protein gene specific for fowl adenovirus (Figure 3).

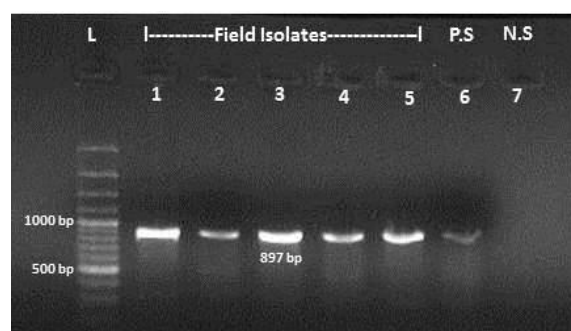


Figure 3: Agar gel electrophoretic picture showing 897 bp PCR amplified product of hexon gene specific for FAdV (L- DNA ladder; 1-5: field isolates/samples, 6 - P.S (Positive Sample), 7 - N.S (Negative Sample)

DISCUSSION

Viral diseases in birds are major threat to the poultry industry as they cause heavy mortality and severe economic loss in terms of production and productivity. These losses may result either due to primary infections by the potentially pathogenic viruses or by secondary opportunistic pathogens. There are many serotypes of fowl adenoviruses (FAdV) that infects chicken but some serotype produces a clinical form of disease, while infection with other serotype may not result

in overt disease particularly in healthy birds. The inclusion body hepatitis–hydropericardium syndrome (IBH–HPS) associated with FAdV has been reported to occur in broilers as well as layers throughout the world (Balamurugan and Kataria, 2004; Asthana et al., 2013). In the past two decades several disease outbreaks have been recorded from poultry flocks of different states and regions of India, causing severe economic losses to the poultry owners and the industry (Dahiya et al., 2002; Gowthaman et al., 2012; Sawale et al., 2012; Kataria et al., 2013; Kumar et al., 2013). The presence of immunosuppressive disease conditions like infectious bursal disease (IBD), chicken infectious anemia (CIA) and mycotoxins prior to infection with FAdV are believed to be the predisposing factor in the pathogenicity of IBH–HPS syndrome (Toro et al., 1999; Toro et al., 2000; Shivachandra et al., 2003; Gowthaman et al., 2012; Hussain et al., 2012). However, several cases of IBH have been reported without any obvious immunosuppressive diseases (Hafez, 2011).

Gross pathological lesions reported in IBH–HPS include an enlarged pale and friable liver sometimes with necrotic foci, ecchymotic hemorrhages, and less consistently ecchymotic hemorrhages can be observed in leg and breast muscles (Meenakshi–Bal et al., 2005). The heart may appear flabby with varying degrees of hydropericardium characterized by presence of a straw–coloured transudate in the pericardial sac. In addition, nephritis, enlarged spleens and thymus atrophy may also be observed in most dead birds (Deepak et al., 2004; Goyal et al., 2009; Hafez, 2011). Most of these gross lesions were consistently found in all the birds examined from the three outbreaks attended during the present disease investigation study. The histopathological lesions observed in the present disease outbreaks were similar and consistently in line with the previous reports by various workers (Kumar et al., 1997; Nakamura et al., 1999; Venkatesha et al., 2005; Amit et al., 2010; Domanska–Blicharz et al., 2011; Hafez, 2011).

The molecular tool of PCR is being widely used to amplify and detect fowl adenoviruses (FAdVs) specific gene in clinical samples for confirmatory diagnosis of diseases associated with these viruses (Ganesh et al., 2002; Parthiban et al., 2004; Rahul et al., 2004; Kataria et al., 2005; Kataria et al., 2013). The primers most commonly used to detect FAdV genome in recent time are set of primers that amplify gene specific for *Hexon* protein with a product size of 897 bp (Meulemans et al., 2001). The present study demonstrated amplification of gene specific for *Hexon* protein in all the clinical samples tested by PCR, representing birds of the three different flocks of the two states confirming the presence of FAdV infection in broilers with apparent hepatitis – hydropericardium syndrome. To conclude, frequent screening and investigation of poultry flocks for fowl adenovirus infection and molecular characterization of FAdV isolates is warranted to identify the subtype(s) of FAdV associated with hepatitis – hydropericardium syndrome in poultry flocks in different parts of India, which would help design appropriate disease prevention and control strategies to counter this virus and its disease outbreaks for alleviating economic losses being suffered by poultry owners and the industry.

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