Isolation, identification and molecular characterization of Inclusion body hepatitis virus

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Abstract

Inclusion body hepatitis caused by avian adenovirus with highly pathogenic strain lead to heavy mortality and unusual lesion like hydropericardium in commercial broilers. Samples were collected from an outbreak in 12000 broiler flock from Andhra Pradesh with sudden high mortality initiated from 32 days of age. Liver was enlarged and showed petechae. Hydropericardium was extensive. 10% liver suspension inoculated to SPF chicks produced mortality with hydropericardium and showed presence of intranuclear inclusions in the hepatocytes. Viral DNA extracted from infected liver processed for PCR produced an amplicon of 890 bp. Nucleotide sequence analysis using blast programme of NCBI showed 99% identity with fowl adenovirus.
Keywords: Inclusion body, Hepatitis, Avian Adenovirus, Broiler, Vaccine, Molecular Characterization.

Introduction

It was earlier believed that the inclusion body hepatitis (IBH) could only be caused by adenovirus if the bird's immune system was first weakened by exposure to immunosuppressive agents such as infectious bursal disease, chicken infectious anemia or mycotoxicosis. Recent work, however, has demonstrated that virulent strains have emerged which alone can produce the severe disease with mortality ranging from 10-30% (Dahiya et al., 2002) which may reach up to 80% (Kumar et al.,2003) in presence of other immunosuppressive factors. The disease is widely prevalent in India and Pakistan and has caused severe economic losses particularly to broiler farmers (Gowda and Satyanarayana, 1994; Memon et al., 2006). Avian adenoviruses vary in their genetic make up, their ability to cause disease, the avian species they affect and the clinical signs they produce. Group-I or conventional adenovirus cause IBH in chicken. Different isolates of the same species and serogroups will vary in pathogenicity and genetic relationship based on PCRF analysis (Erny et al., 1991). It is therefore essential to gene sequence the local isolates for planning effective control measures.

Material and Methods

Investigation was carried out on a 12000 capacity commercial broiler farm in Andhra Pradesh to know the reason for mortality. Age of the broiler flock was 32 days when all of a sudden heavy mortality started. The birds were vaccinated with ND B1 vaccine on 7th day, Gumboro inter mediate plus vaccine on 12th day and ND Lasota vaccine on 18th day. Clinical signs were noted and post mortem lesions were recorded.

Isolation

Liver samples were collected in 20% glycerol saline. A 10 percent suspension (w/v) was prepared by triturating the liver. 0.1 ml of this suspension was inoculated intramuscularly in 10 SPF chicks of 5 days of age. Liver samples were collected from birds that died for PCR and also for histopathological examination.

PCR and sequence analysis

Viral DNA was extracted from infected liver sample using DNeasy Tissue Kit (QIAGEN) as per the manufacturer's protocol. For PCR 2µl of DNA was amplified using 15pmol of each primer (Forward, 5'-CAA RTT CAG RCA GAC GGT -3' nucleotide positions 144-161; Reverse, 5'- TAG TGA TGM CGS GAC ATC AT -3' nucleotide positions 1041-1021). The gene encoding the Hexon protein of fowl adenovirus group-I was chosen for the selection of primers (Meulemans et al., 2001). The amplification was carried out in thermocycler by initial denaturation at 940C for 5min and 35 cycles of 94°C for 2min, 60°C for 1 min and 72°C for 90s, followed by final elongation at 720C for 2min. The amplified product was electrophoretically

separated in a submerged two percent agarose gel and visualized under ultraviolet light. The amplified product was sent to Lab India Private Limited, Gurgaon for sequencing. Obtained nucleotide sequence was analyzed. For further comparison nucleotide sequences of other Fowl Adenovirus isolates were retrieved from the Genbank database (Table: 1). All the nucleotide sequences were aligned for phylogenic analysis using the Clustal W program.

Table: 1. IBH viruses used for comparison

Strain	Accession No.
FADV1	AF339914
FADV2	Af339915
FADV3	AF339916
FADV4	AJ431719
FADV5	AF339919
FADV6	AF339921
FADV7	AF339922
FADV8	AF339918
FADV9	AF508958
FADV10	Af339924
FADV11	AF339920
FADV12	AF339925

Results and Discussions

The clinical signs observed were reduced feed intake, ruffled feathers, depressed appearance, and inability to get up and walk. Deaths during first three days of initiation of outbreaks were 86, 179 and 321 birds. On postmortem, the liver was found enlarged, pale, and friable and in few cases discolored with ecchymosis. In most birds moderate to severe accumulation of straw colored fluid in the pericardial sac leading to hydropericardium was observed (Fig.1). The clinical signs and post mortem lesions observed during present investigation were similar to those described by Gowda and Satyanarayana, (1994), Shane (2000) and Chandra et al.(2000).

On 4th day all the 10 SPF chicks inoculated with suspension prepared from liver homogenate died. Hydro pericardium was observed in 4 chicks while all the chicks showed liver lesions as described for field cases. Histopathologically, liver tissue showed diffuse parenchymatous degeneration with focal areas of necrosis. Many hepatic cells showed presence of basophilic intra nuclear inclusion bodies (Fig.2) which confirmed the disease as inclusion body hepatitis. These lesions were similar to those described in the literature for diagnosis of this disease (Gowda and Satyanarayana, 1994; Shane, 2000; Chandra et al., 2000).

Isolation

PCR and sequence analysis: Sample produced an amplicon of approximately 890 bp as expected with this primer pair by PCR (Fig.3). Using the blast programme of NCBI the obtained nucleotide sequence was found to have 99% nucleotide sequence identity with Fowl adenovirus 4 (FAdV4). On phylogenetic analysis using Clustal W program the virus was found to group with Fowl adeno virus 4 (Fig.4). Meulemans et al. (2001) developed PCR combined with restriction enzyme analysis for detection and differentiation of all 12 fowl adenovirus serotypes and reported FAdV4 among the most frequently isolated virus. Shamim et al. (2009) also carried out similar studies in field out breaks of hydro pericardium syndrome in broilers in Karachi, Pakistan and reported involvement of FAdV4. Dahiya et al. (2002) also confirmed the presence of FAdV4 in such out breaks in Haryana by serum neutralization test and PCR coupled with restriction enzyme analysis.

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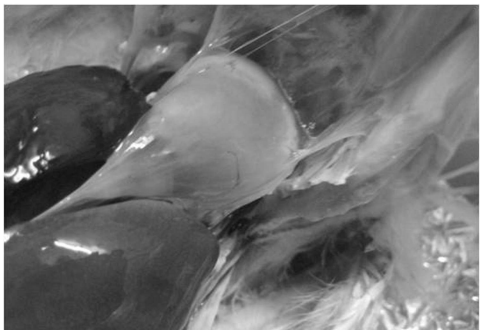

Figure 1. Hydropericardium in field outbreak of Inclusion body Hepatitis

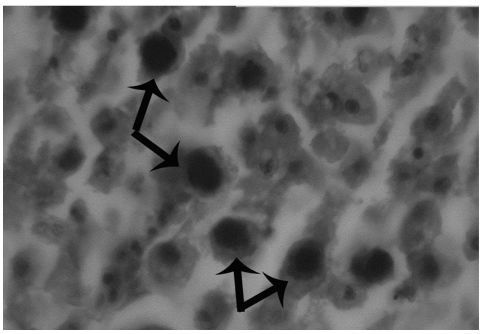

Figure 2. Basophilic Intranuclear inclusion bodies (arrows) in Hepatocytes of infected SPF Chicks

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