Molecular charecterization of Avian Adeno virus causing Inclusion Body Hepatitis-Hydropericardium syndrome in broiler chickens of Anand, Gujarat, India

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Abstract

Avian Adenovirus was isolated from naturally infected broiler chickens. Two Liversample were collected in glycerol saline from the birds came from Aman and Jankipoutry farm for the postmortem in the Dept. of pathology,Veterinary college,Anand(Gujarat). Extraction of viral DNA from infected liver tissues was done as per the method of Meuleman et al., (2001) with minor modifications. The amplified PCR analyzed by agarose gel electrophoresis indicated DNA fragments of approximately 890 bp as expected by primer HexonA F & HexonB R. PCR assay revealed presence of IBH-HPS virus in both samples. Obtained PCR product of both sample were subjected to DNA sequencing and obtained sequencing was compared with other matched sequince. On phylogenetic analysis using Clustal W program showed 3 major group like upper, middle and lower respectively. In the minor branch of upper group the AMAN and JANKI isolates were found to group with Fowl adenovirus 12 strain 380 and Fowl adenovirus 11 strain C2B, so AMAN and JANKI isolates indicating a new fowl adenovirus genotype.

Key Words: Hydropericardium syndrome, Avian Adenovirus, Polymerase Chain Reaction

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Introduction

The avian adenovirus infections are believed to cause heavy economic losses by increasing mortality in chicken, diminished weight gain, poor feed conversion, drop in egg production and poor egg quality. It may also be involved in immuno-suppression leading to increase incidence of secondary infection (Sambrook *et al.*, 1989).

A number of useful methods have been developed for the diagnosis of avian adenovirus infections, including virus isolation in cell culture (Cowen *et al.*, 1978), indirect immunofluo-rescent assay, virus neutralization (Adair *et al*., 1980; Adair *et al*., 1986), enzyme linked immunosorbant assay and the double immune-diffusion (Adair *et al*., 1980; Adair *et al*., 1986; Cowen, 1987). However, most of them are laborious and time-consuming. The main problem with any serological test for adenovirus is associated with the interpretation of results, as antibodies against adenoviruses are commonly found in the blood of both healthy and infected birds. Polymerase chain reaction (PCR) (Saiki *et al.*, 1985) has been applied as a rapid diagnostic tool for the detection of avian viral and bacterial pathogens (Nguyen *et al*., 1994; Ganesh *et al.*, 2002; Dahiya *et al.*, 2002).

This method is not only rapid, but also more sensitive and specific than other diagnostic tests. Utilizing most advanced techniques of molecular biology the hydroperi-cardium syndrome agent can be characterized in order to develop a highly specific vaccine and in turn providing a quick, reliable and specific diagnosis. This study deals with the molecular characterization of avian adenoviruses isolated in Anand Distrtict of Gujarat through a combination of PCR and DNA sequencing.

Materials and Methods

Polymerase Chain Reaction: 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 94°C 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 72°C for 2min. The amplified product was electrophoretically separated in a submerged two percent agarose gel and visualized under ultraviolet light.

DNA sequencing of field isolate: Primers and unincorporated dNTPs present in PCR product of two field sample were removed by PCR purification kit (Perfectprep PCR cleanup 96- cat no. 955156013-Eppendorf). Cycle sequencing was performed following the instructions supplied along with Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The reaction was carried out in a final reaction volume of 20 µl using 200 µl capacity thin wall PCR tube. The reaction was carried out in a final reaction volume of 20 µl using 200 µl capacity thin wall PCR tube. The tubes containing the mixture were tapped gently, spun briefly and then were transferred to thermal cycler. The cycling protocol was designed for 28 cycles with the thermal ramp rate of 100C per second as as Initial denaturation was carried out at 94°C for 5 min followed by 30 sec denaturation at 94°C, 10 sec at 60°C for annealing and 4 min at 72°C for extension. After the reaction, the extension products were purified by using Ethanol-EDTA purification method. Electrophoresis and data analysis was carried out on the automated ABI PRISMTM 310 Genetic Analyzer (Applied Biosystems, USA) using appropriate Module, Basecaller, Dyeset/Primer and Matrix files. The nucleotide sequences of the Hexon protien gene obtained through ABI PRISMTM 310 Genetic Analyzer by using forward and reverse primers were assembled for analysis by SeqScapeV2.5 software programme and a consensus sequence was obtained. Consensus sequence thus obtained was aligned with various published sequences of the Hexon protein gene (Table-1) in Genbank using NCBI Blast and CLUSTAL W (1.82) software programmes. All the nucleotide sequences were aligned for phylogenic analysis using the Clustal W program.

Results and Discussion

Detection of Fowl adenovirus by PCR: The PCR products generated was confirmed for their expected size (897 bp) in 2 % (w/v) agarose gel in 0.5X TBE buffer as per the method of Sambrook et al. (1989) using horizontal submarine electrophoresis apparatus (Bangalore Genei, India). The amplified PCR analyzed by Agarose gel electrophoresis indicated a DNA fragment of approximately 890 bp as expected by primer HexonA F & HexonB R (Fig.1). This was compared with 500 bp ladder marker. No DNA fragments were detected visually in ethidium bromide stained agarose gel electrophoresis when PCR was carried out from DNA extracted from tissues of healthy bird.

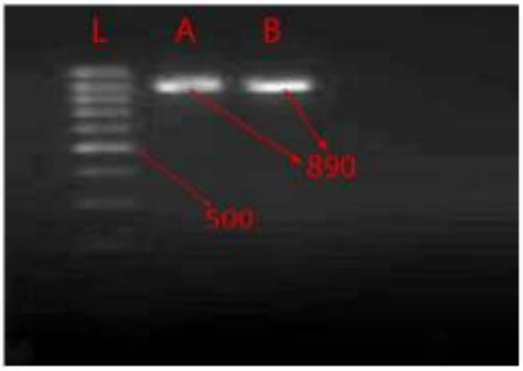

Fig 1. Agarose gel electrophoresis showing PCR amplified product (890) of field isolates.

The similar result was obtained by Meulemans *et al.*, (2001) using same primer. PCR were record by Steer *et al.*,(2009) using same primer of present study of the 12 reference serotypes with the exception of FAdV-5, which

Table-1. IBH virus strains used for comparison (Published sequences)

Strain	GenBank Accession No.	Strain	GenBank Accession No.
FAV1- CELO	AF339914	FAV8- TR 59	AF508956
FAV2- ATCC VR-827	AF339915	FAV9- 764	AF508958
FAV3- ATCC VR-828	AF339916	FAV4- KR5	AF508951
FAV4	AJ431719	FAV2- 685	AF508947
FAV5-ATCC VR-830	AF339919	FAV3- 75	AF508949
FAV6- ATCC VR-831	AF339921	FAV4- 506	AF508950
FAV7-ATCC VR-832	AF339922	FAV5- 340	AF508952
FAV8	AF339918	FAV6- CR119	AF508954
FAV9- ATCC VR-834	AF508958	FAV7- YR36	AF508955
FAV10- ATCC VR-835	AF339924	FAV8- 58	AF508957
FAV11- X11	AF339920	FAV11- C2B	AF508959
FAV12- 380	AF339925	Turkey adenovirus-3(HE virus)	AF074949
FAV5-TR 22	AF508953	Duck adenovirus-1(AAV-EDS)	Y09598
Duck adenovirus-1(AAV-EDS)	Y09598		

Table-2. Matched Fowl adenovirus D Isolates

Sr.no	GenBank Accession no	Fowl adenovirus D Isolates	Matching percentage (%)
1.	EF685519.1	04-52482	94
2.	EF685467.1	04-60057-922	94
3.	EF685579.1	04-40373	95
4.	EF685576.1	04-40372	95
5.	EF685646.1	06-30487	95
6.	EF685526.1	04-52446	95
7.	EF685514.1	04-52487	95
8.	EF685659.1	06-23825-13	94
9.	EF685495.1	04-53357-116	94
10.	EF685491.1	04-53357-120	94

showed low-level or no amplification with the Hexon A/B primer set.

DNA sequencing and phylogenetic analysis of field isolate: Sequencing can be performed on any part of the genome, but was usually performed on a selected part of the Hexon protein gene (Meulemans *et al*., 2001). Using the blast programme of NCBI the obtained nucleotide sequences of both samples(AMAN & JANKI) were found to have 94% identity with fowl adenovirus 11 isolate 1047 hexon protein gene(Accession no DQ323984.1), 94% identity with Fowl adenovirus 11 isolate FAdV-11/Brazil/2006/USP-0 (Accession no Fj3607 47.1) and also Fowl Adenovirus D of different isolates show different percentage of identity with AMAN and JANKI isolates that shown in Table no.2.

For comparison nucleotide sequences of other Fowl Adenovirus isolates were retrieved from the Genbank database shown in Table no.2. Nucleotide sequences of the Hexon protien gene of the isolates from Aman P.F. and Janki P.F. were aligned with other isolates in the Clustal W program. Phylogenetic analysis of nucleic acid sequence was done with foreign isolates and tree diagram created (Fig.2).

On phylogenetic analysis using Clustal W program showing 3 major group like upper, middle and lower respectively (Fig.2). Within the uppers group, 2 minor branches were observed. In the minor branch of upper group the AMAN and JANKI isolates were found to group with Fowl adenovirus 12 strain 380 and Fowl adenovirus 11 strain C2B. However, within this major upper group, a minor branch was formed by AMAN and JANKI isolates indicating a new fowl adenovirus genotype. FAV6-CR119 Strain could not be assigned to any clusters within groups and might be a representative third cluster (Lower group). The different strains like FAV11-X11, FAV7-ATCC, FAV9-764, FAV7-YR36 etc. are involved in the minor branches of middle group.

The sequencing of sample M158-04 was carried out by Raue *et.al*., (2005) and the overall percentage of identity among the sequences obtained from the FAdV reference strains and M158-04 ranged between 60.3 to 67.0%, whereas

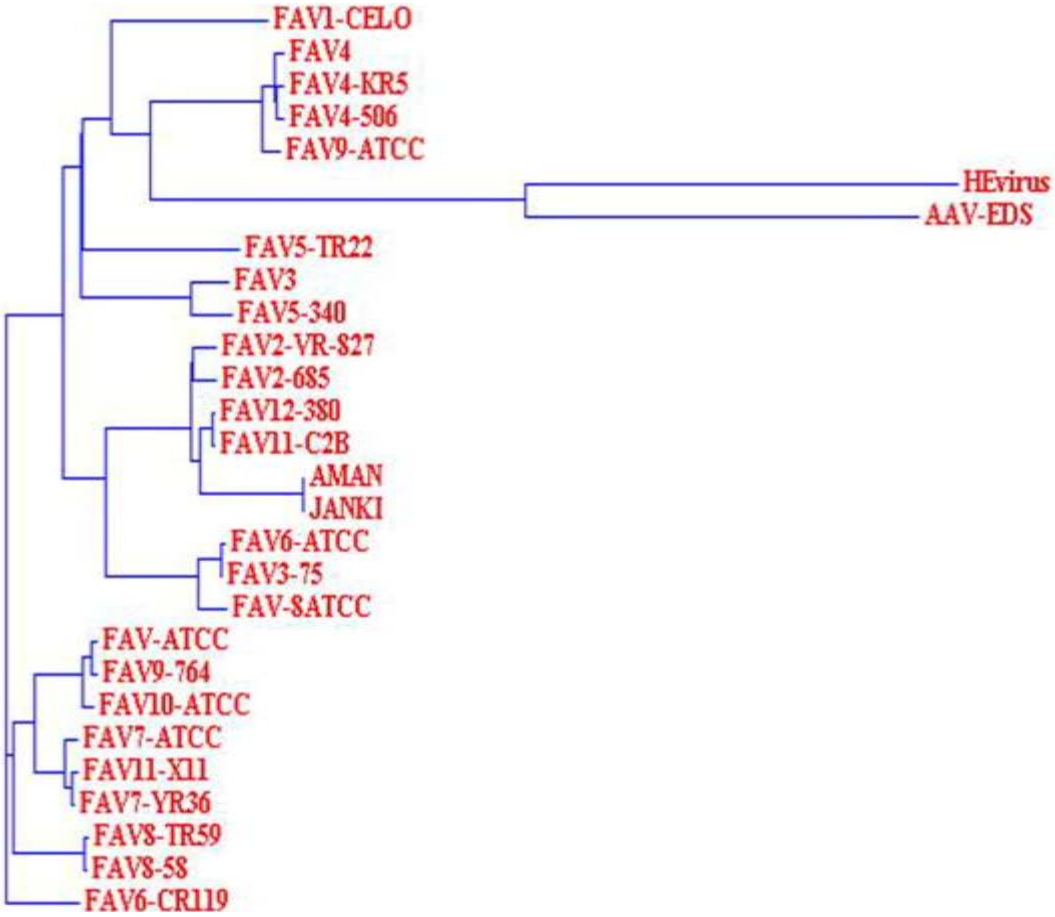

Figure-2 Phylogenetic tree based on nucleotide sequnces of Hexon Gene of field isolates and sequnces from Gene bank.

that of the TAdV3 sequence and M158-04 was only 14.1%. With 12.5%, the sequence of EDSV showed the lowest percentage of identity when compared with that of M158-04. A phylogenetic tree was established, showed 3 major branches for the proposed genera, Aviadenovirus (FAdV1–12), Atadenovirus (EDSV) and Siadenovirus. Within the Aviadenovirus branch, 7 minor branches were observed. However, within this major branch a sole minor branch was formed by M158-04 indicating that M158-04 represents a new avian adenovirus genotype.

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Conflict of interest

Authors declare that they have no conflict of interest.

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