Scientific Research Publishing

American Journal of Molecular Biology, 2020, 10, 246-258
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

PCR Based Detection and Phylogenetic Analysis of Fowl Adenovirus Strains Isolated from 2019 Epidemic from Punjab and Sindh, Pakistan

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How to cite this paper: Sharif, N., Mehmood, M.D., Naqvi, S.Z.H., ul-Haq, H.A., Ahmed, S.S., Ghani, M.U., Shoaib, M. and Hussain, M. (2020) PCR Based Detection and Phylogenetic Analysis of Fowl Adenovirus Strains Isolated from 2019 Epidemic from Punjab and Sindh, Pakistan. *American Journal of Molecular Biology*, **10**, 246-258.
https://doi.org/10.4236/ajmb.2020.103016

Received: April 29, 2020
Accepted: July 18, 2020
Published: July 21, 2020

Abstract

Hydro-Pericardium Hepatitis (HPH) is an emerging infectious disease of commercial poultry, caused by different serotypes of Fowl Adeno Virus. The vertical transmission of the virus into the progeny may results in devastating damage, causing huge economic losses to its farmers. In present study, molecular typing is performed on basis of partially conserved hexon gene sequences, using a unique set of primers having common reverse oligo for simultaneous detection of FAdV1, FAdV-4 and FAdV-11. A total of 14 fowl adeno virus strains were isolated from 100 suspected adeno virus liver samples, collected from different districts in Pakistan, between 2018 and 2019. FASTA's sequence alignment and phylogenetic analysis revealed that out of the 14, one isolate which belonged to group A showed 27% similarity with FAdV-1, while three isolates showed 99%, 95% & 45% similarity to FAdV-4 (Group C). Whereas, ten isolates showed more than 99% similarity to FAdV-11 (Group D). The serotypes FAdV1, FAdV-4 and FAdV-11 are prevailing in the breeder and broilers. These results hold great importance in rapid, reliable and simultaneous detection of the three FAdV serotypes. Therefore, fowl adeno virus vaccine production for commercial poultry shall be according to the prevalent field serotypes.

Keywords

Hydro-Pericardium Hepatitis (HPH), Hexon Gene, Polymerase Chain Reaction, Phylogenetic Analysis, Sanger's Sequence

1. Introduction

Infectious hepatitis is a common problem of modern commercial poultry farming in Pakistan. It is caused by a variety of toxic and infectious agents, which include feed toxicity, bacteria and mostly harbored by influenza and adeno virus subtypes. Fowl adeno virus subtypes are the most common cause of Hydro-pericardium Syndrome (HPS), Hydro-pericardium Hepatopathy Syndrome (HHS), Hydro-pericardium Hepatitis Syndrome (HHS), Inclusion Body Hepatitis (IBH) and Egg Drop Syndrome (EDS) [1] [2].

Fowl Adenovirus (FAdV) belongs to the family Adenoviridae and genus Aviadenovirus divided into 5 species (FAdV-A to FAdV-E) based on their restriction enzyme digestion pattern and into 12 serotypes on the base of cross neutralization test [3] [4]. It has a double-stranded DNA genome of approximately 43 to 46 kb, which encodes 10 major structural and 11 non-structural proteins, including E1A, E1B, DBP, ADP, E4, 52/55 K, pIVaII, pol, EP, 33 K, and 100 K [5] [6]. Hexon gene is the major protein, which controls the variability of FADV, and has the sequence of 2800 bp to 2900 bp with the weight of 103 Kda resulting diversity of 12 serotypes. Its mortality rate ranges from 30% to 70% in broilers and significant drop in egg production in breeders and layers [7] [8] [9]. FAdV spread both horizontally by faeces and vertically in the mean of progeny.

In 1987 the disease was first identified at Angara Goth near Karachi in Pakistan. HPS and IBH are contagious viral problems of breeders and fast-growing broilers are being infected particularly at young age. The disease is characterized by off feed and water, respiratory rales, pasting of whitish colored diarrhea on vent and morbidity up to 100%. Fowl adeno virus infections are conventionally diagnosed by sign symptom, appearance of typical lesions, virus isolation on *in vitro* tissue culture and chicken embryonated eggs. Agar gel precipitation test (AGPT) is still being used for the detection of fowl adeno virus antibodies in exposed birds, whereas, indirect enzyme linked immunosorbent assay (ELISA) and virus neutralization test (VNT) are other reliable tools for detecting type-specific antibodies and groups. Due to the high diversity in the FADV strains, diagnosis of FADV strains has become very expensive and time-consuming process in Pakistan. The attempt was made with the aim to produce a novel technique for the rapid identification of three different FADV serotypes in a single PCR reaction using common reverse oligo. This would help in saving time and cost for rapid screening of commercial breeder and layer flocks in future.

2. Materials and Methods

2.1. Sample Size

The sample size was calculated by using the formula of Kish & Lisle states that [10]

$$n = Z^2 P(1 - P)/d^2$$

*w*here, z = Score for 95% confidence interval = 1.96;

P = Prevalence (To estimate the proportion of morbidity with HPS & IBH);

d = Sampling error that could be tolerate = 5%;

$1P$ = Probability.

2.2. Sample Collection

Using statistical formula ($n = Z^2P(1 - P)/d^2$) 30 g of liver were collected in sterile polythene labelled bag from each of 100 suspected Adenovirus infected broilers/breeder flocks reported from all across Pakistan and transported to Ottoman Pharma (Immuno Division) where it was stored at −20˚C till further use. The detailed history of these collected isolates was described in Table 1.

2.3. Virus Isolation

10% of liver homogenate was prepared in 0.9% Normal saline (Zeesol Pharma-Pakistan) solution in an electric homogenizer (B. Braun-Germany). The suspension was centrifuged at 2˚C and 3000 g for 2 minutes and the supernatant was collected for filtration. The clear supernatant was then passed through a 0.2 µm size filter (Sartorius-Germany) and admixed with 1% of pen strep (Gibco-USA) in the final concentration.

Table 1. History details of isolates recovered from different flock all across Pakistan.

Sr. No.	Sample Name	Source	Flock type	Post Mortem lesions	Morbidity	Mortality
1.	D14.EDS/CHICKEN/OP.PK/2019	Rawalpindi	Broiler	Digestive	8%	25%
2.	D13.HPS/CHICKEN/OP.PK/2019	Gujarat	Breeder	Respiratory	5%	20%
3.	D12.HPS/CHICKEN/OP.PK/2019	Gujranwala	Broiler	Digestive	15%	40%
4.	D11.HPS/CHICKEN/OP.PK/2019	Lahore	Broiler	Digestive	6%	22%
5.	D10.IBH/CHICKEN/OP.PK/2019	Karachi	Broiler	Digestive	10%	50%
6.	D09.IBH/CHICKEN/OP.PK/2019	Pattoki	Broiler	Digestive	1%	18%
7.	D08.IBH/CHICKEN/OP.PK/2019	Sheikhupura	Breeder	Respiratory	5%	30%
8.	D07.IBH/CHICKEN/OP.PK/2019	Sahiwal	Broiler	Digestive	5%	30%
9.	D06.IBH/CHICKEN/OP.PK/2019	Gujranwala	Breeder	Respiratory	5%	30%
10.	D05.IBH/CHICKEN/OP.PK/2019	Gujranwala	Broiler	Digestive	7%	22%
11.	D04.IBH/CHICKEN/OP.PK/2019	Lahore	Breeder	Respiratory	10%	38%
12.	D03.IBH/CHICKEN/OP.PK/2019	Karachi	Broiler	Digestive	10%	35%
13.	D02.IBH/CHICKEN/OP.PK/2019	Rawalpindi	Broiler	Digestive	5%	30%
14.	D01.IBH/CHICKEN/OP.PK/2019	Lahore	Broiler	Digestive	2%	25%

*Digestive signs—loose dropping, whitish Pasting or vent, Mucoid droppings due to excess bile acids; *Respiratory signs—gasping, sneezing, pulmonary edema, lethargy huddling.

2.4. Virus Preparation on Cell Lines

1.5% solution of Dulbecco's Modified Essential Medium (DMEM) (Gibco-USA) was prepared in doubled distilled water. Vero cell line was retrieved from liquid nitrogen and one million cells were added to the T-25 tissue culture flask (SPL-Germany) supplanted with 10% Fetal Calf Serum (FCS) (Gibco-USA). Antibiotic combination of penicillin and streptomycin was added at 1% concentration. The cells containing roux flask was incubated at 37˚C for 48 hours in the presence of 5% CO_2.

2.5. Virus Isolation and Propagation

T-25 tissue culture flask containing Vero cell line confluent monolayer was infected with 0.5 ml of filtrate liver homogenate. 2% FCS was added in the flask and incubated at 37˚C for 48 - 72 hours. The monolayer was observed for cytopathic effect after every 12 hours and results were recorded.

2.6. DNA Extraction

The flask containing the Infected Vero cell line was freeze at −40˚C for half an hour and then incubated at 37˚C in a water bath for 10 minutes. The process was repeated three times and the cell media suspension was centrifuged at 3000 g for five minutes. Sediment was decanted and the supernatant was collected in the sterile tube that is subjected to DNA extraction. The supernatant was collected and modified as described by QIAGEN-Germany.

2.7. Oligos Designing

Partial CDS sequences of the Hexon gene of all 12 FADV serotypes were cited from the Gene bank database with the help of their accession numbers (AF339914 to AF339925). All the 12 sequences were aligned on the bioinformatics tool (Clustal Omega 1.2) for multiple sequence alignment and similarity. Serotype 1, 4 and 11 showed the maximum similarity index of more than 75%. So, these three sequences were separately aligned again to find out the conserved region for designing of highly sensitive primers. Common reverse primers were designed from lower strand exonic part of the L5 loop region of the Hexon gene. Similarly, other 3 forward primers were designed from the upper exonic region of Hexon gene. Serial cloner 1.3 was used to run the PCR in silico to check the efficacy of primers before order and further their Amplicon sizes were estimated as mention in Table 2.

Table 2. Primer sequences, amplicon size and melting temperature in accordance with genotype.

SR.	Primer	Sequences	Amplicon Size	TM Value (˚C)	Genotype
1	Forward	CAGCGGGCGTGAAGCA	1219 bp	55	Strain 1
	Reverse	TAGTGATGACGGGACATCAT		56	
2	Forward	CGAGGATTACGACGATTA	900 bp	52	Strain 4
	Reverse	TAGTGATGACGGGACATCAT		56	
3	Forward	CGGCGCCCGGACAAAA	899 bp	55	Strain 11
	Reverse	TAGTGATGACGGGACATCAT		56	

2.8. Polymerase Chain Reaction (PCR)

Three positive sense primers along with common negative primers were used in the reaction of multiplex PCR against 1, 4 and 11 strain. One microliter of the forward primer of each serotype along with one microliter of common reverse primer for all three serotypes was added into the 100 µl PCR reaction tube. Other ingredients of the recipe include 12 µl master mix, 7 µl injection water, and 4 µl of each processed DNA sample. The PCR programmed was set at initial denaturation at 95˚C for five minutes followed by 45 cycles of denaturation at 95˚C for 45 Sec, annealing at 55˚C for 45 Sec and extension at 72˚C for the 90 Sec. The final extension was done at 72˚C for 10 minutes. 2% agarose gel (Bio world) was prepared following method recommended by Shahzad [11].

2.9. Sanger's Sequencing

50 µl of PCR product (Amplicon) for each liver sample was organized according to the Performa and submitted in the advance bioscience international for dispatch to Korea. The sequencing was performed by the Sanger method.

2.10. Phylogenetic Analysis

After sequencing, all the 14 sequences were annotated (NCBI Annotation v5) to find out their CDS regions. All our sequences were directly submitted to the NCBI database. Alignment and phylogenetic reconstructions were performed using the function "build" of ETE3 v3.1.1 as implemented on the GenomeNet (https://www.genome.jp/tools/ete/) [12]. Alignment was performed with MAFFT v6.861b with the default options [13]. The tree was constructed using Fast Tree v2.1.8 with default parameters. Values at nodes are SH-like local support to find out the likelihood between all the strains.

3. Results

On the request of farmers, different broiler/breeder farms were visited in the Punjab province & Pakistan. The Adenovirus suspected dead birds showed whitish vent pastings and most of them were found in dorsal recumbency. On postmortem clear straw color fluid varies from one ml to five ml in few birds was recorded but the liver was enlarged showing uniformly distributed petechial hemorrhages throughout each lobe. The liver was highly fragile and pale in color with longitudinal and vertical whitish streaks as showed in **Figure 1**. There was mild splenomegaly, nephritis, and severe hepatitis also recorded in some birds.

Vero cell line confluent monolayer showed vacuolation and detachment of cells after 72 hours of infection under 20× magnification lens of inverted microscope (Nikon-Japan) as depicted in **Figure 2**. The viral harvest showed more than $1 \times 10^{6.2}$ tissue culture infective $dose_{50}$. Polymerase chain reaction for each cultivated samples showed variable amplicon size in gel electrophoresis. Fowl Adeno type 1, type 4 and type 11 showed amplicon size of 1216, 900 and 900 bp respectively as showed in **Table 3**.

Table 3. FAdV group allocation after successful cultivation on cell line and PCR based confirmation.

Sr. No.	Source	Flock Type	Sample Showing Typical Factors	Growth on Vero Cell Lines	PCR Results (Amplicon size bp)	Sequencing Results
1.	Rawalpindi	Broiler	EDS	YES	1216	Group A
2.	Gujarat	Breeder	HPS	YES	900	Group C
3.	Gujranwala	Broiler	HPS	YES	900	Group C
4.	Lahore	Broiler	HPS	YES	900	Group C
5.	Karachi	Broiler	IBH	YES	900	Group D
6.	Pattoki	Broiler	IBH	YES	900	Group D
7.	Sheikhupura	Breeder	IBH	YES	900	Group D
8.	Sahiwal	Broiler	IBH	YES	900	Group D
9.	Gujranwala	Breeder	IBH	YES	900	Group D
10.	Gujranwala	Broiler	IBH	YES	900	Group D
11.	Lahore	Breeder	IBH	YES	900	Group D
12.	Karachi	Broiler	IBH	YES	900	Group D
13.	Rawalpindi	Broiler	IBH	YES	900	Group D
14.	Lahore	Broiler	IBH	YES	900	Group D

*HPS (Hydro pericardium syndrome), *IBH (Inclusion body hepatitis).

Figure 1. Pathognomonic lesions of IBH.

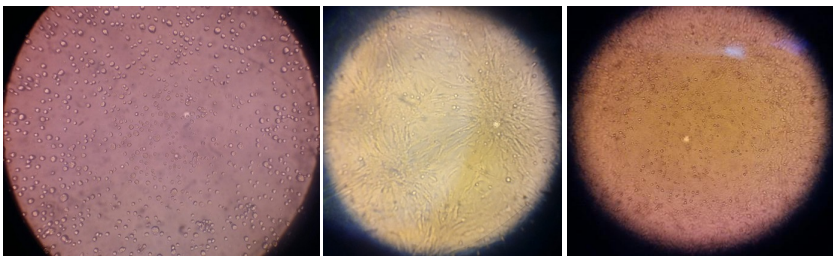

Figure 2. Normal and FAdV infected Vero cell line.

The sequencing of each PCR product revealed that three different types of FAdV were present in the infectious material. The dominant type was FAdV-11 (75%) followed by FAdV-4 (60%) and FAdV-1 (10%). The FASTA sequences alignment with the partial coding DNA sequences (CDS) regions taken from local and Austrian gene banks showed partial homology with the few samples whereas, the non-homologous pattern for most of the DNA sequences. 90% of samples were declared positive based on sign symptoms whereas, 70% were reported in PCR amplification as depicted in **Figure 3**.

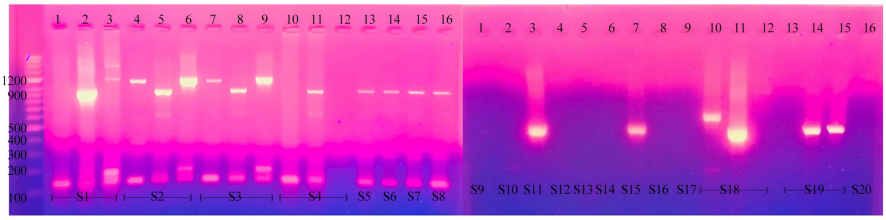

Figure 3. Gel electrophoresis.

The chromatogram for hexon gene of fowl adeno virus nucleotide specificity in sequencing is shown in **Figures 4-6**, whereas, similarity index of isolates with reference fowl adeno viruses by NCBI blast along with generated accession number has been depicted in **Table 4**.

Hexon Gene sequence of FAdV-1 (Group A):

Locus: D14.EDS/CHICKEN/OP.PK/2019 852 bp DNA linear VRL 20-DEC-2019

Organism: Fowl Aviadenovirus A

1 caaccanatc caaccgagaa ggccagaggc tccagatcag gttttacccg acgcagaccg
61 acgacacgcc caacagttac cgcgtgcggt acagcttaaa cgtgggtgac agttgggttc
121 tggacatggg agccacctac ttcgacatca agggcgtcct agacagagga ccttctttta
181 aaccgtatgg aggaaccgca tacaatcccc tcgcgccccg cgaagccttt ttcaacaatt
241 gggttgacac agaggcgagc aagaccgtca tcacgggtca gatgacaact ccctacgaaa
301 acgtccaggg cgctaaagac aagactgccg cgatcgtcgc cgctctttca ggggtttatc
361 ccgatcccaa tatcggtacc gccatcagcg agatgggcgc cttaaacgcg acgtcggcag
421 cccaagtcgg attggctgcc cgattcgcga aagtatcgag cgataacacg cgtctagcct
481 acggagccta cgttaaaccg ctcaagaacg acggttctca atcgattaac cccactcctt
541 actgggtcat ggacagcaac gccacaaact atctcggagt catgggagtc gaagacttta
601 gcgcctcgct aacctatccc gatacgctcc ttattccccc gccaaccgaa tactcagaag
661 tgaataccgg cgtcatgaag gcaaacaggc cgaattacat cggatttagg gacaatttta
721 tcaacctgct ctatcatgat acgggtgtgt gctcgggtac tctgaattcg gagcgttcgg
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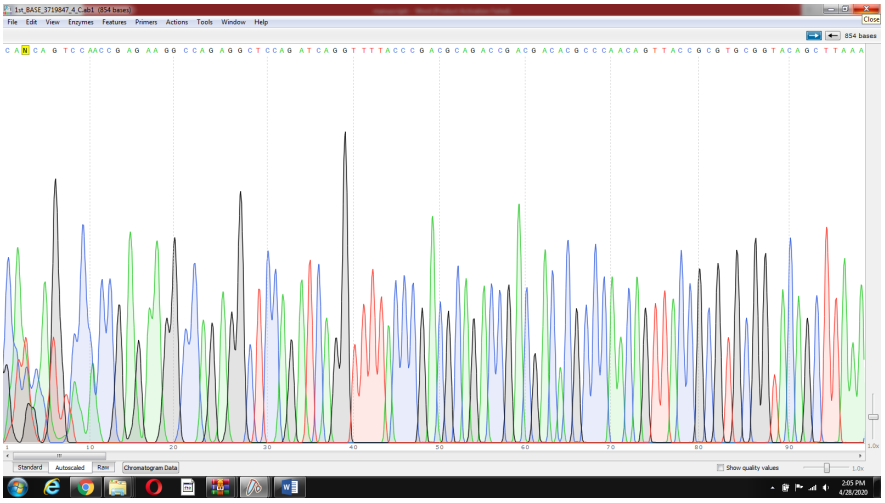

Figure 4. Chromatogram of FAdV-1 (Group A) nucleotides specificity in sequencing.

Hexon Gene sequence of FAdV-4 (Group C):

Locus: D11.HPS/CHICKEN/OP.PK/2019 874 bp DNA linear VRL 20-DEC-2019

Organism: Fowl Aviadenovirus C

1 cggcacccta acccaccgag gaaggccaga ggctccagat caggttttac ccgacgcaga
61 ccgacgacac gcccaacagt taccgcgtgc ggtacagctt aaacgtgggt gacagttggg
121 ttctggacat gggagccacc tacttcgaca tcaagggcgt cctagacaga ggaccttctt
181 ttaaaccgta tggaggaacc gcatacaatc ccctcgcgcc ccgcgaagcc tttttcaaca
241 attgggttga cacagaggcg agcaagaccg tcatcacggg tcagatgaca actccctacg
301 aaaacgtcca gggcgctaaa gacaagactg ccgcgatcgt cgccgctctt tcaggggttt
361 atcccgatcc caatatcggt accgccatca gcgagatggg cgccttaaac gcgacgtcgg
421 cagcccaagt cggattggct gcccgattcg cgaaagtatc gagcgataac acgcgtctag
481 cctacggagc ctacgttaaa ccgctcaaga acgacggttc tcaatcgatt aaccccactc
541 cttactgggt catggacagc aacgccacaa actatctcgg agtcatggga gtcgaagact
601 ttagcgcctc gctaacctat cccgatacgc tccttattcc cccgccaacc gaatactcag
661 aagtgaatac cggcgtcatg aaggcaaaca ggccgaatta catcggattt agggacaatt
721 ttatcaacct gctctatcat gatacgggtg tgtgctcggg tactctgaat tcggagcgtt
781 cgggtatgaa cgtcgtcgtc gagctccagg acagaaacac ggaacttagt taccagtaca
841 tgttagccga tatgatgtcc ggtccttcac taaa

Figure 5. Chromatogram of FAdV-4 (Group C) nucleotides specificity in sequencing.

Hexon Gene sequence of FAdV-11 (Group D):

Locus: D1.IBH/CHICKEN/OP.PK/2019 1099 bp DNA linear VRL 20-DEC-2019

Organism: Fowl Aviadenovirus D

1 ctgaaggttc catatccagg ttccgcagaa atacttcgct attaaaaatc tgctgctgtt
61 gcccggcacc tacacctacg agtgggtgct cagaaaggac cccaacatga ttctgcagtc
121 cagtttaggc aatgatctga gagccgacgg agcttcgatc gtgtactctg aagtaaacct
181 aatggctaac ttcatgccca tggatcacaa tacaagcaat cagcttgagc tcatgctcag
241 aaacgccact aacgatcaaa cattcgcaga ctatctaggt gccaagaatg cgttgtatca
301 agtacccgcg ggctccacgg cacttaccat taacattcca gctcgcacct gggagggtat
361 gcgtggttgg tcttttaccc gtgtcaaagc ttcagaaact ccgcagatag gagcccaata

421 tgacgttaac ttcaagtatt cgggaacaat tccatactct gacggcacct tctatttgac
481 ccacacgttc aggaacatga gcgtgctatt cgacacgtcc atcaattggc ccgggaacga
541 ccggttgcta gcacccaacc tgttcgaaat caagcgcaat gtgggcatcg attcagaagg
601 gttcacgatg tcccaatgtg acattactaa agattggtac ctgatccaaa tggccaccaa
661 ctacaactac gtatttaacg gataccggtt ctggcccgac agacaatatt tccattatga
721 cttcctccgg aatttcgacc ccatgacccg gcagggccct aatttccaag accaaacatt
781 gttcgattta accgaatacg aacccacgat acccatctct ccaggatcta tgcaaacggg
841 gcaagacgct atccgtaata attcaggcta cacagctccg cgcagttggc ccgtatacag
901 cgctcagcag gatgagtctt ggcccgccaa ttggccttat ccgctcatcg ggagcgaatc
961 catcctgccg tcccanatgg ttaactacaa gaagttcttg ggcgacaact atctatggac
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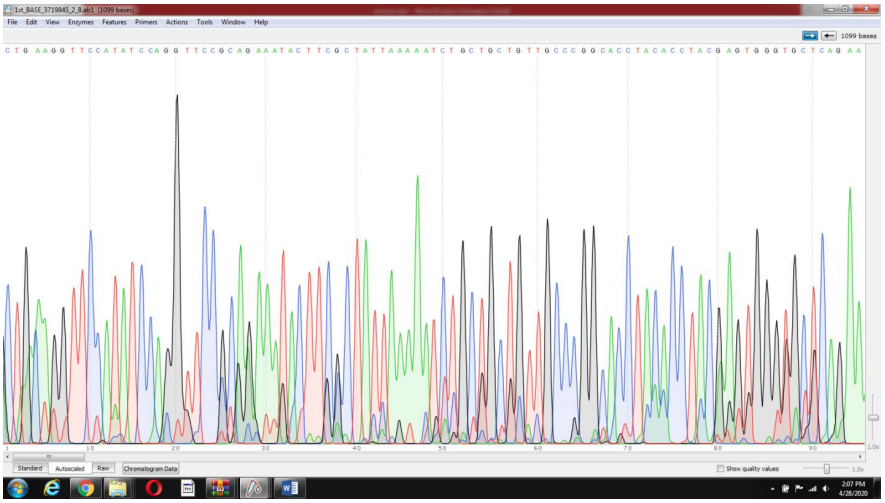

Figure 6. Chromatogram of FAdV-11 (Group D) nucleotides specificity in sequencing.

Table 4. NCBI accession number, similarity index and genotype by using blast.

Isolate Accession No.	Gen Bank Accession No.	Percentage Similarity%	Serotype Groups	Genotype
MN861105	MK575871.1	27%	Group A	FAdV 1
MN861104	MK572871.1	45%	Group C	FAdV 4
MN861103	MH151202.1	99%	Group C	FAdV 4
MN861102	MK875248.1	95%	Group C	FAdV 4
MN861101	MH379252.1	99%	Group D	FAdV 11
MN861100	MG029114.1	99%	Group D	FAdV 11
MN861099	MH379254.1	98%	Group D	FAdV 11
MN861098	MH379251.1	98%	Group D	FAdV 11
MN861097	MK572871.1	99%	Group D	FAdV 11
MN861096	MH379251.1	99%	Group D	FAdV 11
MN861095	MH379252.1	99%	Group D	FAdV 11
MN861094	MK572871.1	98%	Group D	FAdV 11
MN861093	MK572871.1	99%	Group D	FAdV 11
MN861092	MK572871.1	99%	Group D	FAdV 11

*Prevalence of FAdV (FAdV-1 7%, FAdV-4 21%, and FAdV-11 78%).

The L5 loop region of the Hexone gene sequences of isolates from Punjab and Karachi regions were submitted in GenBank with their accessions numbers (MN861092 to MN861105) respectively. The isolate number MN861105 showed 27% similarity with the accession number MK575871 as compare to the isolate numbers MN861103 have 99% sequence similarity to the accession numbers MH151202. Similarly, three isolates MN861099, MN861098 and MN861094 have 98% sequence similarity to accession number MH379254, MH379251 and MK572871. Based on sequencing similarity percentage to the FAVs reference strains and phylogenetic analysis revealed that 71% of isolates were FAV-11 followed by FAV-4 (21%) and FAV-1 (7%).

4. Discussion

The sequencing of FAdV PCR products obtained from a unique set of designed primers confirmed the presence of three fowl Adenovirus isolates from the different geographical areas of Pakistan. The isolates were cultivated on the Vero cell line and confirmed the progeny through PCR using three different sets of primers. The sequencing of these products confirmed FAdV1, FAdV4, and FadV11, whereas other serotypes such as FAdV-2, FAdV-6, and FAdV-12 had been reported for its circulation in the environment [14]. However, in the current study, only three subtypes have been confirmed in which FAdV-11 is the dominant strain that has to be considered the major factor causes huge losses in the recent outbreaks.

The results of the current study corroborate with the findings of Deherdt who reported that HHS (Hepatitis Hydro pericardium syndrome) cases linked to FAdV-4 whereas IBH (Inclusion Body Hepatitis) cases are associated with FAdV-8 and FAdV-11. FAdV-1 has also been isolated from many farms in which birds showed remarkable gizzard atrophy [15] [16] [17]. Concurrent infection of FAdV with multiple types is common [18].

Pathological findings such as Hepatitis, Hydro-pericardium Syndrome, Inclusion Body Hepatitis, and severe pleural ascites along with fibrinous air vasculitis were observed in almost every case we studied. Several birds from different farms were recorded positive for chicken anemia viruses and infectious bursal disease as well. Five farms were also infected with Newcastle disease virus; sever tracheitis and inflammation of the upper respiratory tract is very well exploding on post mortem. De-Herat reported that chicken anemia virus/infectious bursal disease virus are predisposing and supportive factors for augmentation of inclusion body Hepatitis [15].

The results of the analysis indicated that all the isolates belong to the family adenoviridae and their sequences showed a high resemblance to the sequences of loop L1 of Adenovirus strains recovered from commercial and wild birds. A total of 63 FAdV references and reported FASTA sequences of 14 FAdV reference and reported strains were downloaded from the GenBank database including 5 Pakistanis and 4 ATCC cultures. According to nucleotide sequence alignment and construction of phylogenetic tree one strain was categorized in species FAdV-A;

three strains were grouped into FAdV-C; and the remaining 12 strains were placed into FAdV-D. The Hexone gene is the longest gene of the Adenovirus genome responsible for antigenic and immunogenic properties [19] [20]. Phylogenetic tree structure consists of three main branches representing FAdV Spp as FAdV-A, FAdV-C, and FAdV-D. The first clad originated from branch showed the 27% similarity of isolate MN861105 with the reference strain MK575871, whereas isolate MN861093, MN861094 were showing 45% and 99% similarity with the MK572871 and MH151202. When the 2nd clad wad observed our fourth strain MN861096 was showing a 95% similarity index with the MK875248 of reference strains. In the third clad of our phylogenetic tree our 10 sequences MN861095, MN861097, MN861098, MN861099, MN861100, MN861101, MN861102, MN861103, MN861104, and MN861105 were showing 99% and 98% ratio with the NCBI reference strains MH379252, MG029114, MH379254, MH379251, MK572871, MH379251, MH379252, MK572871, MK572871, and MK572871 respectively as mentioned in Figure 7.

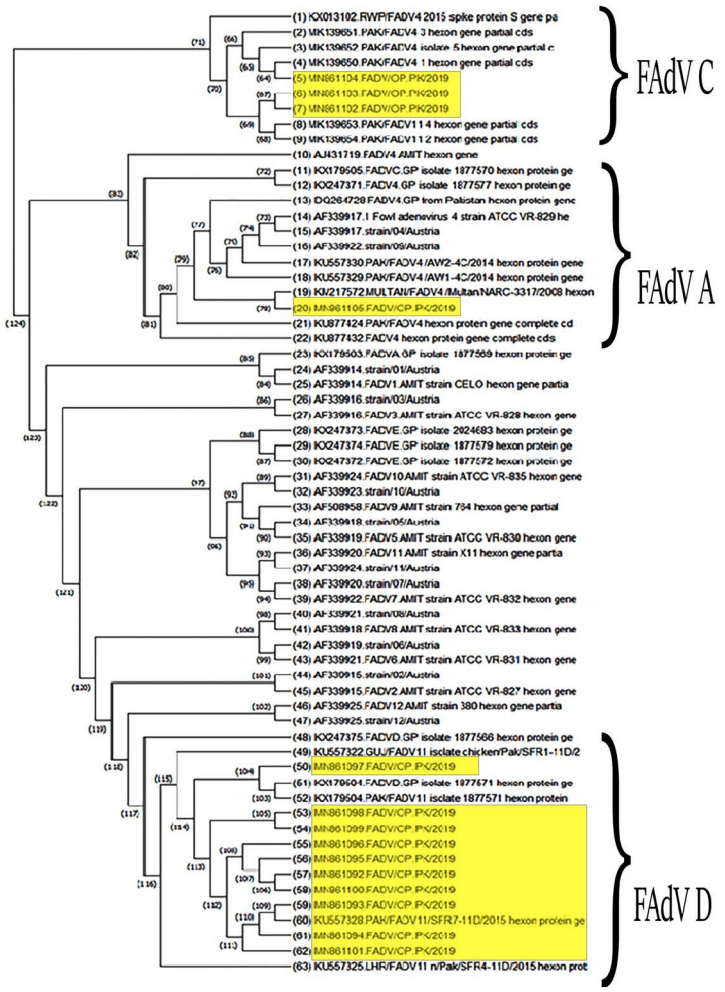

Figure 7. Phylogenic analysis of the indeginous fowl adeno virus strains based on partial coding DNA sequences of hexon gene deanalysed indeginous strain are highlighted. 1 strain belongs to specie A/FAdV-1, 3 isolates belong to specie C/FAdV-4 and 10 strains belong to specie D/FAdV-11.

Highest prevalence of FAdV-11 (71%) is followed by FAdV-4 (21%) and FAdV-1 (7%). Our results are similar to results from Toro where Adenovirus isolates frequently belong to the spp Aviadenovirus D group [21]. The serotype of FAdV-1 was least frequent (8%). Moreover, FAdV serotypes FAdV-3, FAdV-6, FAdV-9, and FAdV-10 have not been reported in Poland. The difference in the L1 sequence of the Hexon gene of FAdV within the clad and even in group may be due to the mutation in the HVR-1 (Hexon variable region). This difference could also be observed between the isolates from different countries and continents.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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