SEQUENCING AND PHYLOGENETIC ANALYSIS BASED ON HYPERVARIABLE (LOOP 1) REGION OF HEXON GENE OF PAKISTANI ISOLATES OF AVAIN ADENOVIRUSES, FROM IBH-HPS CASES IN 2015

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ABSTRACT

Molecular techniques coupled with bioinformatic analysis has been shown to be a rapid, cost-effective and efficient strategy for diagnosis and serotype characterization of fowl adenoviruses. The study involved diagnosis and characterization of fowl adenoviruses from the inclusion body hepatitis-hydropericardium syndrome cases in Pakistan in 2015. Fowl adenovirus DNA was isolated and hexon gene region was amplified by PCR and amplicons were sequenced by Sanger dideoxy sequencing. A total of 8 adenovirus hexon gene loop 1 region sequences were used in subsequent phylogenetic analysis with the previous isolates identified from BLAST. Serotype 11 was diagnosed in 87% and serotype 1 in 10% of the cases while the least identified serotypes were serotype 8 (4%) and serotype 4 (2%). Sequences were submitted to NCBI. Query sequences together with the respective homologues were retrieved in FASTA format and imported to MEGA 7.0 for multiple sequence alignment using clustal-w tool. Neighbor joining method was used to infer evolutionary history and 1000 bootstrap replicates were applied. Tajima-Nei method was selected to compute evolutionary distances, shown as number of base substitutions per site. Exclusion of alignment gaps resulted in 487 positions in the final dataset and 32 sequences were used to build the phylogenetic tree. Results of bioinformatics analysis indicated close relationship of the query sequences with the previous isolates of fowl adenoviruses from Pakistan, India, China, Austria, Itlay, Russia, US and Canada.

INTRODUCTION

- Adenoviruses belonging to the family Adenoviridae possess a linear genome of double stranded DNA, are non-enveloped, and have icosahedral symmetry.
- Fowl adenoviruses ubiquitously infect a huge number of birds causing inclusion body hepatitis, quail bronchitis, gizzard erosion, hydro-pericardium syndrome, and pancreatic necrosis.
- Such infections in poultry chickens lead to heavy economic losses due to high mortality rate, poor weight gain, poor feed conversion and meager egg production. Additionally, adenovirus infection may lead to immuno-suppression, thus increasing the risk of secondary infection.
- Hydro-pericardium syndrome, which has a sudden onset, and generally has mortality rate up to 75%, is characterized by transparent or straw-colored fluid in the pericardial sac, along with nephritis, hepatic necrosis and intra-nuclear inclusion body formation in eosinophils or basophils within hepatocytes.
- The applications of molecular diagnostics methods like Polymerase chain reaction and restriction digestion has gathered significance, being sensitive and rapid techniques, allowing detection of viral DNA as well as identification and typing, which is very important to control the disease.

OBJECTIVES

- Analyzing the variants of adenovirus in Pakistan, affecting the broiler chicken with inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) in 2015.
- Processing of liver samples of infected chicken for DNA isolation and PCR amplification of hexon gene region.
- Characterization of serotypes by RFLP analysis and Sanger dideoxy sequencing.
- Multiple sequence alignment of obtained sequences with homologues identified from BLAST.
- Building Phylogenetic relationship based on the sequence alignment of loop1 of hexon gene.

MATERIALS AND METHODS

1. Sample Collection
2. DNA Extraction
3. PCR Amplification
4. Amplicon Purification and RFLP Analysis
5. Sanger Dideoxy Sequencing
6. Phylogenetic Analysis

RESULTS

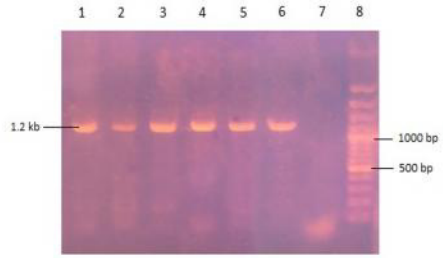

Figure I: Agarose gel electrophoresis of amplified product (hexon gene) from adenoviral DNA using H1 and H2 primers. Lane 1-6: Amplified product, Lane 7: Negative control and Lane 8: 100 bp DNA marker (SM0323)

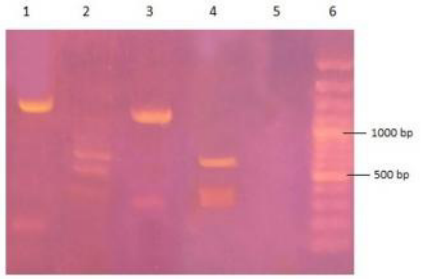

Figure II: Restriction with the enzyme BfoI to determine RFLP, in Agarose gel electrophoresis, of the amplified product for identification of serotypes. Lane 1: serotype 4, Lane 2: serotype 1, Lane 3: serotype 8, Lane 4: serotype 11, Lane 5: negative control and Lane 6: 100 bp DNA marker (SM0323)

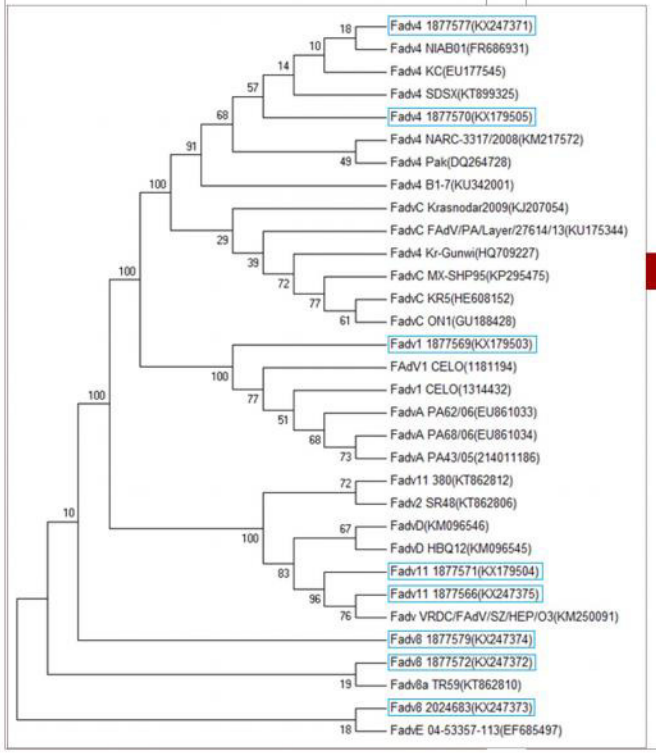

Figure III: Phylogenetic tree constructed using NJ method based on the sequence alignment of hexon gene loop 1 region. MEGA 7.0 was used for evolutionary analyses. Those sequenced in this study are highlighted in color with their submitted NCBI accession numbers.

CONSLUSION

This strategy based on PCR coupled with RFLP, sequence analysis is an efficacious way for identification and study of fowl adenovirus isolates. Commercial broiler flocks should be routinely tested by PCR for FAdV infection screening and for ensuring proper vaccination prior to infection and disease prognosis. Further, molecular studies can be done to determine the severity of infection caused by Pakistani isolates of FAdVs and to ensure the efficiency of currently available vaccines in controlling any future disease outbreaks in Pakistan.