



Detection and identification of avian adenovirus in broiler chickens suspected of inclusion body hepatitis in Khuzestan, Iran during 2015-2016

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Keywords

Avian adenovirus, broiler, FADV-11, IBH, Southwest Iran

Abstract

Avian adenoviruses (AAV) are known as a very diverse group of pathogens causing a variety of clinical symptoms or being totally asymptomatic in poultry flocks. The aim of this study was the molecular detection of avian adenoviruses in broiler flocks suspected of the IBH and respiratory syndrome in the southwest of Iran. For this intent, the liver and lung samples with macroscopic lesions were collected from 30 different poultry flocks (poultry of slaughterhouse and flock mortalities). Subsequently, DNA was extracted from samples and examined using PCR. The L1 (Loop1) region of the hexon gene was amplified. PCR products were sequenced to reveal the identity of the avian adenoviruses. The data resulted from the nucleotide sequencing were analyzed using programs and services provided by National Center for Biotechnology Information (NCBI). The results showed that the pools of liver samples from a 25 days old flock were positive in the PCR test. Based on the sequence data, adenoviruses belonged to the D genotype of avian adenoviruses. In phylogenetic analysis, FADV isolates were closely related to the FADV-11 isolates of Iran, China, Canada and Australia with nucleotide homology up to 99%. This is the first study on molecular detection and analyzing the nucleotide sequence of hexon gene fragment of FADV in broiler farms in Southwest Iran.

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Abbreviations

AAV: Avian Adenovirus
IBH: Inclusion Body Hepatitis
L1: Loop 1
FADV: Fowl Adenovirus
bp: Base Pair

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Introduction

Adenoviruses cause a wide range of infections in the chickens all around the world. Infected farms might show high mortality rates, liver lesions (IBH), hydropericardium syndrome (HPS) and secondary infections caused by suppression of immune system. Several adenovirus strains led to lymphocyte depletion in bursa, thymus and spleen which in turn suppress the immune system [1]. Adenoviruses are categorized into four genera: Mastadenovirus (in mammals), Aviadenovirus (in birds), Adenovirus (in birds, mammals and reptiles) and Siadenovirus (in birds and amphibians) [2]. The International Committee for Taxonomy of Viruses (ICTV) proposed twelve serotypes for aviadenoviruses classification [3]. Already, FAdV known strains have been alienated into five genotype species, including A to E genotypes [1]. There exists a correlation between the FADV genotypes and serotypes. Genotype A includes FAdV1, type B: FAdV5, type C: FAdV4 and 10, type D: FAdV2, 3, 9 and 11 and genotype E contains FAdV6, 7, 8a and 8b [4]. Inclusion body hepatitis (IBH) is a significant disease in 3 to 6-week-old broiler chickens. Various serotypes of fowl adenoviruses have been detected in natural occurrences of IBH, especially those in E genotype can cause serious liver damage in IBH [5].

Currently, PCR is a rapid and sensitive technique developed for identification of avian adenoviruses. For the purpose of identifying and assigning isolates to the serotypes and species, molecular methods, especially PCR, have been being generally used [5]. The hexon (NCBI accession number: MG738474) is known as one of the major structural proteins of fowl adenoviruses. This protein is determinative for type, groups and subgroup-specific indexes. The hexon gene was chosen as the target for primer preparation in molecular diagnostic test [1]. With reference to the past studies carried out in Iran, no study has reported avian adenoviral infections in the Southwest of Iran. The aim of this study was detection and identification of avian adenoviruses in broiler chickens suspected to inclusion body hepatitis (IBH) and respiratory syndrome in Southwest Iran during 2015-2016.

Results

The PCR with degenerate primers resulted in the amplification of a 590 bp product, as expect-

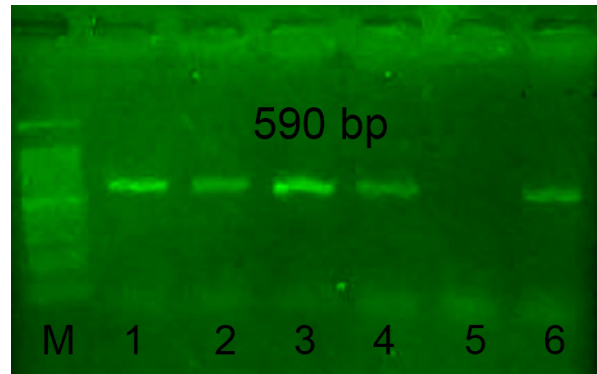


Figure 1

Electrophoresis analysis of PCR products from amplification of avian adenovirus (AAV) gene: Lane M = marker 100 bp, lane 6= positive control (590 bp), lane5= negative control, lane 1-4 = positive samples.

ed. The results showed that one 25 days old flock with 10% mortality (3.3%) was positive for AAV (). A phylogenetic tree (Figure 2) was constructed based on the nucleotide sequences of the L1 hexon gene and the corresponding regions of the other AAV strains rescued from GenBank. Phylogenetic analysis of this isolate revealed that they could be classified into 11 serotypes of D genotype of avian adenoviruses (). Furthermore, this is closely related to the previously reported AAV isolates such as KF406339.1 (Iran), KU981146.1, ANG57898.1, KU981145.1, KU981143.1 (China) and EF685644.1 (Fowl adenovirus D Canada) with nucleotide homology which reached up to 99%.

Discussion

Inclusion body hepatitis (IBH) has been recognized as an economically important disease occurring throughout the world [6,7,8,9]. Different serotypes of FAdV are able to cause IBH disease. In the last decade, most of the isolated serotypes which are responsible for IBH, belong to D and E genotypes [10]. In this study, the infection rate of AAV was 3.3%, which indicated the limited distribution of AAV in Ahvaz region. Nateghi et al. (2014) reported that prevalence of avian adenoviruses in broilers in northeast Iran was 10% [2]. Herdt et al. (2013) in a ten-year survey, detected Fowl adenovirus infections in 38 of 310 diseased Belgian broiler flocks [1]. Based on the L1 loop of hexon gene sequence analyzing, the IBH prevalence is usually related to the serotype 6, 7 and 8; However, the viruses correlated with those outbreaks in Iran were adenovirus serotype 11 [11]. The nucleotide sequence data of the obtained pos-

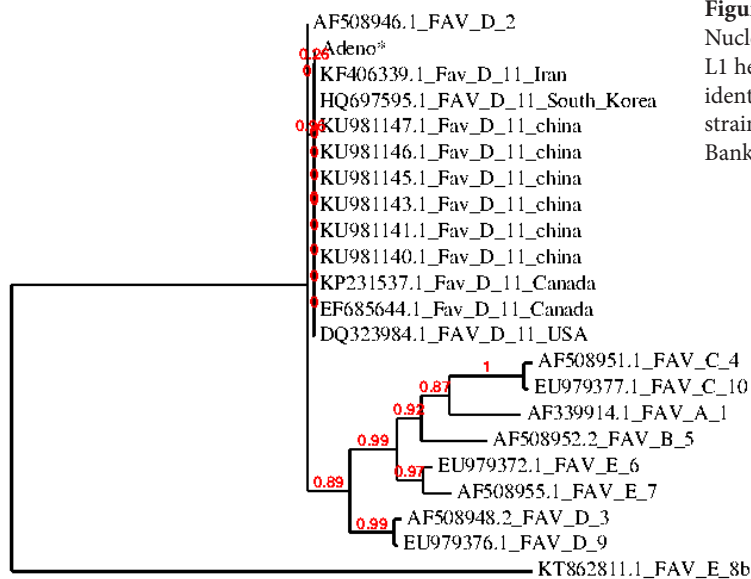


Figure 2
Nucleic acid-based phylogenetic relationships, of L1 hexon protein gene of one avian adenovirus identified from Ahvaz, Iran and the 21 reference strains. The sequences were obtained from GenBank. FAdV isolate in this study is marked with *.

itive sample showed that this adenovirus belonged to FAdV-11, which is in the D genotype group. Hoseini et al. (2012) identified FAdV-11 serotype from mortalities of three-week-old chicken farms [5]. They concluded that this serotype is able to cause clinical diseases and mortality in chickens. In Nateghi et al. (2014) study, FAdV 8b, 2 & 11 were identified [2]. In Herdt et al. (2013) survey, FAdV isolates belonged to the serotypes FAdV 1, 2/11, 3, 5 and 8a [1]. FAdVs correlated with IBH

prevalence by hexon gene loop 1 sequencing in Canada from 2000 to 2006 were genetically linked to FAdV2, 8a and 11 [12]. Again, in 2003, the Fowl Adenovirus isolates in Ontario, Canada matched with Serotype 11 in group D genotype [13]. FAdV-11 strain 1047 was the reason for IBH outbreaks in Saskatchewan, Canada [12]. Another study in 2007 identified 18 FAdV-11 amongst 55 fowl adenovirus from different flocks in South Korea with 99% homology to 1047 strain [14]. In 2010, Fowl adenovirus serotype 11 found in Hungary [15]. Alvarado et al. (2007) recognized isolated adenovirus as Stanford strain and characterized as European Serotype 9 [16]. Since most cases of IBH are the consequences of the vertical transmission, vaccines have been proved to be a highly successful solution for controlling IBH by preventing vertical transmission and inducing maternal immunity [16,17].

In conclusion, this study confirms the presence of solely serotype 11 aviadenovirus in broiler flocks in Southwest Iran. The rate of AAV detection in broiler chicken flocks around Ahvaz was inconsiderable. As a result, detection of aviadenovirus showed that AAV is not prevalent in respiratory disease and liver lesions in Ahvaz area.

Materials and Methods

Samples

The poultry of slaughterhouse having hepatic and respiratory liver and lung involvements were evaluated. The liver and lung samples were taken from 20 different flocks; Each flock was compris-



Figure 3
A liver showing petechiae and ecchymosis signs, as well as being enlarged and pale.

ing 10,000 chickens, 10 chickens with liver and lung lesions were selected from each flock for taking samples. In addition to this, liver samples (which were enlarged, pale, showing petechiae, and ecchymosis signs) (Figure 3), were taken from 10 to 35 days old broiler chickens of 10 farms located around Ahvaz. These farms were experiencing increasing mortality (10 to 30%). Five to 10 liver samples of each farm were combined as pools. Finally, 410 samples were collected and included in this study.

It should be noted that the adenovirus vaccines are not common in broiler chickens in Iran.

DNA extraction

The DNA extraction from the tissue samples was performed with the Cinnapure DNA extraction kit (Cinnaclon Co., Iran). 50-100 mg of each tissue sample was cut into small pieces using a scalpel and placed into a sterile 2 ml microtube. In order to complete the homogenization process, 100 μ l of prelysis buffer and 20 μ l of ributinae were added to the microtube and it was kept at 55°C for 3 hours. During incubation time, the microtube was vortexed (for 5 seconds) at 5 minutes intervals to increase the DNA yield. Stages of the DNA extraction were carried out carefully according to the manufacturer's instruction. Finally, extracted DNA was collected in a sterile 1.5 ml microtube and stored at -80°C for the subsequent PCR process.

Polymerase Chain Reaction (PCR)

The 590 bp region of the hexon gene (NCBI accession number: MG738474) was amplified using a pair of specific primers. The said primers were Hex L1-F (5'-ATGGGAGCSACCTAYTTC-GACAT-3') and Hex L1-R (5'-AAATTGTCCCK-RAANCCGATCTA-3') (2). The PCR process was carried out in a 20 μ l reaction volume containing 10 μ l 2x Ampliqon PCR master mix (Denmark) with 1.5 mM MgCl₂, 0.5 μ l of each primer (10 Pmol/ μ l), 6 μ l of ddH₂O and 3 μ l extracted viral DNA. As such, the thermocycler (Quanta Biotech, Germany) was configured in the following conditions: 94°C for 5 minutes followed by 35 cycles of 94°C for 60 seconds, 54.2 °C for 45 seconds, 72°C for 60 seconds, and a final step at 72°C for 5 minutes. Meanwhile, in all stages of PCR, negative control (ddH₂O instead of DNA) and positive control (the DNA from Australian FAdV-8b vaccine strain (Intervet Pty Ltd.)) were utilized. The PCR products

(590 bp) were separated by electrophoresis using 1% agarose gel. The RT-PCR products were cut from the gel, purified by the AccuPrep PCR Purification Kit (BioNeer Co., South Korea) according to the manufacturer's procedure, and then sequenced in the forward direction. Sequencing reactions were performed by BioNeer Co., South Korea.

Characterization

In order to perform genotyping, a 590bp region of the hexon gene of the extracted positive DNA sample was amplified. Then, the nucleotide sequence was aligned and compared with those of previously identified isolates from Iran and worldwide avian adenoviruses references. The nucleotide sequences of the hexon gene determined in this study were also compared to the AAV sequence data available in the National Center for Biotechnology Information database (<http://ncbi.nlm.nih.gov>) using nBLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), and the phylogenetic relationship was established by http://www.phylogeny.fr/simple_phylogeny.cgi. All of the sequences were aligned with the use of CLUSTAL W. Distance-based neighbor joining trees were constructed using the approximate likelihood-ratio test (aLRT) available in http://www.phylogeny.fr/simple_phylogeny.cgi.

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Author Contributions

Molecular detection of virus: Z.B., P.T.G.; Diagnosis of affected poultry carcasses: A.R.; Scientific counseling: M.M.; Sampling: S.E.

Conflict of Interest

Conflicts of interest: none

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