The detection of fowl adenovirus in chickens with hydropericardium syndrome in Isfahan and Charmahal-Va-Bakhtiyari provinces, Iran

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Abstract

The hydropericardium in chickens is most common in Isfahan and Chcharmahal-va-Bakhtiyari, Iran. For this, the study was achieved for investigation of adenovirus role in induction of hydropericardium in these regions. In this study, 20 broiler flocks suspected to hydropericardium were sampled from hydropericardium fluid. Then, DNA was extracted and amplified by specific primers. The amplified fragment for detection of AV was 896 bp. The results showed that 10 in 20 flocks, and 47 in 200 samples were positive to AV. The results revealed that most of infected flocks were more than 30 days old. In this study, the correlation between infectivity to adenovirus and husbandry system were not significant. The infectivity rate in Isfahan and Chaharmahal-va-Bakhtiyari was not significant. Therefore, in addition to high altitude, adenovirus infection can play a role in increasing the prevalence of hydropericardium in these areas.

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Introduction

Fowl Adenovirus (FAdV) belong to the genus of *Aviadenovirus* that have been associated to a number of disease conditions including Inclusion Body Hepatitis (IBH), Hydropericardium Hepatitis Syndrome (HPS), Gizzard Erosions (GE), proventriculitis and tenosynovitis.[1-3] This virus, like Mark's disease virus, chicken anemia virus and Gamboro disease virus, can suppress the immune system.[4] The immunosuppression is a common property of most adenoviruses infection in chickens that is manifested by lymphocytic depletion in bursa, thymus and spleen.[1]

Adenoviruses are categorized into 4 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 *Aviadenovirus* classification, based on serum neutralization test. Already, FAdV could be classified into five species, based on molecular structures, including A to E genotypes.[3] 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.[1] Serotype 4 which is a member of the species FAdV C has been implicated in HPS.[2]

HPS is caused by hydropericardium and hepatic necrosis. HPS was also known as Angara Disease: the first outbreak was observed in Pakistan in 1987,[1] and it was subsequently reported in many countries, including most middle east countries, resulting in significant economic losses to poultry industry.[3] HPS is typically observed in 3 to 5 weeks of age in chickens and is characterized by mortality range from 30% to 70%.[2] The main lesions of HPS are hepatitis and hydropericardium.[5]

Until now, HPS has been reported in many countries, including Iraq, Kuwait, India, Mexico, Ecuador, Peru, Chile, USA, Russia, Japan, and Poland, resulting in considerable economic losses.[3] The clinical and gross lesions of HPS in broilers in Iran has been showed but so far there is no scientific report relating to adenoviral HPS. So, PCR technique as a specific and rapid technique for detection of FAdV was utilized in this study for the identification of fowl adenovirus in HPS cases in broiler chickens.

Materials and Methods

Collection of samples

Two-hundred liver samples were collected from 20 commercial and traditional broiler flocks in Charmahal-va-Bakhatiyari and Isfahan provinces. Tissue samples were collected from suspected flocks based on clinical and necropsy finding of HPS.

DNA extraction

DNA was extracted from samples with commercial extraction kit (DNP, Cinagen, Iran), according to the manufacture· manual.

Polymerase Chain Reaction (PCR)

PCR was carried out in a total volume of 50 µL containing extracted DNA, 20 pmol of each primer, 200 µmol DNTPS, 1.5 µmol $MgCl_2$ and 2 U Taq polymerase (Fermentas, Iran). The primer sequences were: F: 5CAARTTCAGRCAGACGGT3 and R: 5TAGTGATGMCGSGACATC3[1]

The PCR was carried out in thermal cycler (Eppendorf, Germany) with initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 58°C for 60 sec, with final extension at 72°C for 7 min. The PCR product was analyzed in 1.0% agarose gel. In this study we used double distilled water (ddH_2O) as negative and fowl adenovirus vaccine (Intervet Ltd) as positive control. The PCR was optimized for amplification of fowl adenovirus hexon gene for generation 896 bp PCR product.

Results

The PCR product of 896 bp was observed in positive control and 47 of the 200 field samples (23.5%; Figure 1). No amplification was seen with DNA of negative control.

Overall, 10 flocks from 20 flocks were infected with adenoviruses. The age range in infected broiler flocks was 28 to 56 days old. These flocks were reared in traditional or commercial growing systems, but there was no significant difference between two growing systems with respect to percentage of infectivity.

The analysis of data revealed there are no significant differences between different areas in Charmahal-va-Bakhtiyari and Isfahan province, for infectivity of adenovirus.

Discussion

Fowl adenoviruses have been incriminated as etiological agents for a number of clinical conditions in broilers. Among the diseases caused by FAdVs, IBH and HPS are economically important. Almost all serotypes of FAdVs have been reported to cause IBH in broilers,[2] while only FAdV-4 causes HPS. Routinely, HPS outbreaks are diagnosed on the basis of clinical signs, gross pathological lesions, histopathology and/or agar gel precipitation test.[6,7] The PCR has been noted to be a rapid sensitive and specific test to detect avian adenovirus infection.[6] The genome of FAdV-4 encodes a number of nonstructural proteins and three structural proteins: hexon, penton and the fiber protein. The hexon gene of FAdVs is the longest and consists of hypervariable Loop L1 (HVR1-4) regions, making it a hotspot for research on classification and antigenic shift of FAdVs. Hexon protein is the main target for induction of serotype-specific neutralizing antibodies.[3] The hexon (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 PCR test.[1]

Until now outbreaks of FAdVs has been frequently reported worldwide. Over the past 5 years, epidemics with mixed serotypes have been observed in different regions, such as FAdV-2, -11, -7 and -8 in Europe and North America,[6] FAdV-4 in Asia[8] and FAdV-2 and -8b in South Africa.[7] The most important disease associated with FAdVs infection in chickens are Inclusion Body Hepatitis (IBH), Hydropericadium Hepatitis Syndrome (HPS), Gizzard Erosion (GE).[1]

HPS has been recognized as an economically important disease in the world.[9] In this study the infection rate of FAdV in Isfahan and Charmahal-Va-Bakhtiyari province was 23.5%. The first report of FAdVs infection in Iran investigated the prevalence of avian adenovirus in northeast Iran broiler chickens.[7] They reported 10% infectivity rate due to FAdVs in chickens that is close to our results.[7] Furthermore, there are some reports of FAdVs infection worldwide. Herdt *et al.* (2013) detected FAdVs infection in 38 of 310 diseased Belgian broilers flocks.[10] In this study predominant serotype of FAdVs infection was not investigated based on previous reports; some different serotype was reported from Iran. Hossini and Morshed identified FAdVs-11 serotype from dead 3-week-old broiler chicken flock and showed that this serotype caused clinical disease and mortality in this flock.[11] Also Natghi *et al*. detected 2, 8b and 11 serotypes in broiler chickens.[7] Furthermore, Ghafari *et al.* isolated serotype 11 in group d genotype based on nucleotide sequence of hexon gene fragment of FAdVs in broiler farms in southwest Iran.[8] These viruses were genetically linked to FAdVs 2, 8a and 11, which matched with serotype 11 from Ontario and Canada.

Since the primers used in this study can amplify DNA from all the 12 FAdV serotypes, it is unlikely that the negative results in some samples were due to PCR performance. Another possibility is that the viral load in the samples was below the detection limit.[11] In the field, certain disease conditions are associated with occurrence of HPS. The HPS outbreaks in broiler chicks and some other birds have been reported to be associated with mycotoxicosis and drug toxicities.[11] In this study the identification of serotypes was not applied. According to previous studies the common serotype in HPS infection in broilers can be serotype 4.[6] By considering that commercial fowl adenovirus vaccine contained FAdV-4, the utilization of available vaccine can induce full protection against challenge with this serotype.

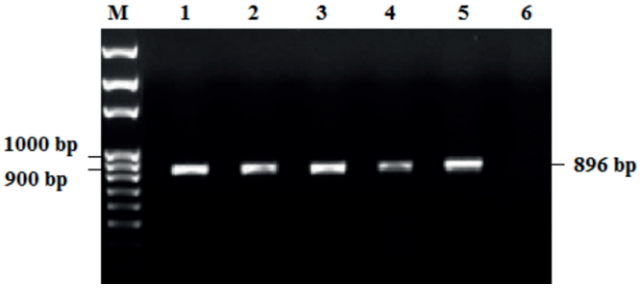

Figure 1. Gel electrophoresis of FAdV hexon gene amplification (M, marker; 1, positive control; 2 to 5, positive samples; 6, negative control).

Different attempts have been made to control outbreaks of FAdV. Unfortunately, commercial vaccines with veterinary council approval are currently not available in the Iranian market and only a few vaccines against HHS are available in the other countries. Up to now, three types of vaccines, inactivated vaccine, oral live attenuated vaccine, and recombinant vaccine against HHS and IBH have been reported.[12] The preparation of the inactivated vaccine is easier and faster than both the live attenuated and recombinant vaccines. Several scholars have already reported that HHS and IBH can be effectively controlled through the use of inactivated vaccines.[5,13,14] The oil emulsion inactivated FAdV-4 vaccine is a promising candidate, providing effective heterologous protection. Efficacy studies using a virulent virus challenge showed that a single dose of oil emulsion inactivated FAdV-4 vaccine in broilers and could provide excellent clinical protection against both homologous and heterologous challenge. HHS was not observed in any of the FAdV-4 challenged immunized chickens.[5]

Conclusions

In conclusion, according to prevalence of FAdVs infection in broiler flocks in Iran, vaccination against this infection is proposed in broiler breeder for induction and transmission of suitable maternal antibody for successful controlling of FAdVs infection in broiler chickens.

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