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Short Paper

Molecular detection of fowl adenovirus 7 from slaughtered broiler chickens in Iran: the first report

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Abstract

Background: Fowl adenoviruses (FAdVs) are responsible for a variety of clinical symptoms, with an increasing significance in the poultry industry throughout the world. Typical diseases caused by FAdVs include inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), gizzard erosion (GE), respiratory disease, and hemorrhage in muscles and organs. **Aims:** During 2020, broiler chickens from the north of Iran showed ecchymotic and petechial hemorrhages in thigh and breast muscles at the slaughterhouse. Hemorrhages were observed in 10% to 60% (with an average of 20-30%) of chicks per flock. To find out the etiology of these lesions, the present study was conducted. **Methods:** Different environmental factors were investigated, and FAdV, infectious bursal disease virus (IBDV), and chicken infectious anemia virus (CIAV) were detected using molecular assays. **Results:** Among the viruses tested, FAdV was detected by polymerase chain reaction (PCR), and sequence analysis clustered the virus into species E, serotype 7. **Conclusion:** This is the first report on FAdV-7 existence among poultry in Iran. Effective screening of the chicks at slaughtering age should be performed from the whole country.

Key words: Broiler, Fowl adenovirus, Hemorrhage, *Hexon* gene

Introduction

The family Adenoviridae contains five genera of *Atadenovirus*, *Adenovirus*, *Ichtadenovirus*, *Mastadenovirus*, and *Siadenovirus*. There are 14 species at the genus *Adenovirus* infecting fowl, duck, falcon, goose, pigeon, turkey, and psittacine. Out of the 14, 5 species are classified into fowl adenoviruses (FAdVs), A-E species is responsible for a variety of clinical symptoms (Kaján *et al*., 2019). Typical diseases caused by FAdVs include inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), gizzard erosion (GE) (Kaján *et al*., 2019), respiratory disease (Ren *et al*., 2019), and hemorrhage in muscles and organs (Kefford *et al*., 1980).

The five species of FAdVs are further divided into 12 serotypes (FAdV-1 to 8a and 8b to 11) based on cross-neutralizing tests (Chen *et al*., 2019). A serotype or species can be further categorized into genotypes (Kaján *et al*., 2013). Molecular methods such as polymerase chain reaction (PCR) are suitable alternatives for conventional methods of diagnosis since they enable the comparison of the results with available gene sequences. The gene coding the major capsid protein, the hexon, and viral DNA polymerase are the targets of PCR assays to detect FAdVs among which, the variable loop region of the *hexon* gene is usually used for FAdV typing (Kaján *et al*., 2013).

Broiler chickens from four farms of Gorgan city, Golestan province, Iran, showed ecchymotic and petechial hemorrhages in thigh and breast muscles at the slaughterhouse (Fig. 1).

Materials and Methods

Sample collection

From April 2020 to May 2020, broilers transported from four farms to Gorgan slaughterhouses showed hemorrhages in their muscles. From chicks showing the signs, 20 chicks were selected for sampling. They were at the age of slaughter, and different tissue samples from the thymus, liver, and bursa of Fabricius (totally 60

samples) were collected in terms of FAdV, infectious bursal disease virus (IBDV), and chicken infectious anemia virus (CIAV). This article does not contain any studies with human participants or animal performed by any of the authors.

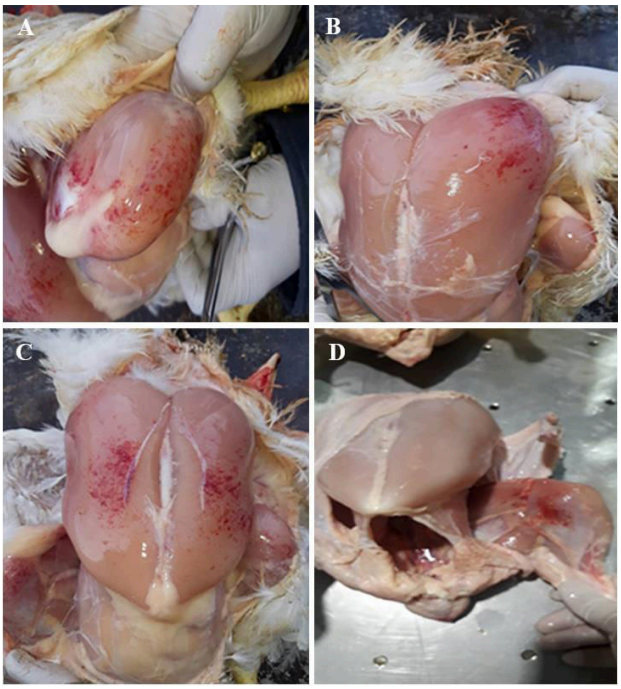

Fig. 1: Ecchymotic and petechial hemorrhages in the thigh and breast muscles of broilers in Gorgan slaughterhouse. (**A**) Petechia in lateral part of thigh muscle, (**B**) Unilateral hemorrhages in breast muscle, (**C**) Bilateral hemorrhage in breast muscle, and (**D**) Petechia in medial part of thigh muscle

DNA and RNA extraction

Tissue samples were homogenized using a mortar and pestle. The DNA/RNA was extracted using the SinaClon DNA/RNA extraction kit (SinaClon, Tehran, Iran).

CIAV detection by PCR

The VP1 part of the CIAV genome was amplified using the forward primer of 5´-AGC CGA CCC CGA ACC GCA AGA A-3´ and reverse primer of 5´-ATC AGG GCT GCG TCC CCC AGT ACA-3´ (Hiremath *et al*., 2013).

IBDV detection by reverse transcription-polymerase chain reaction (RT-PCR)

cDNA was synthesized using the Thermofisher cDNA synthesis kit. The RT-PCR was developed by Sapats and Ignjatovic (2002) using primers J1 (5´-GGC CCA GAG TCT ACA CCA TAA C-3´) and J2 (5´-CCG GAT TAT GTC TTT GAA GCC-3´) to amplify *VP2* gene (Razmyar and Peighambari, 2009).

FAdV detection by PCR

A previously designated PCR was used to amplify a 590-bp region of the hexon gene (Steer *et al*., 2009).

FAdV sequencing and phylogenetic analysis

The amplified 590-bp region of the *hexon* gene of FAdV was analysed via electrophoresis and ultraviolet (UV) imaging. The amplicons were gel purified (GeneJET gel extraction kit, Fermentas) and sent for sequencing. Sequencing was performed in both directions by an ABI 3130 sequencing machine (Bioneer, Korea). The sequenced data was compared with the loop region of the hexon gene sequences of different FAdVs retrieved from GenBank. Multiple sequence alignments were generated using ClustalW, the alignments were subsequently used to construct distance matrices using the Kimura 2-parameter model implemented in Mega7 software. Neighbor-joining (NJ) trees were plotted with Mega7 with a 1,000-fold bootstrap approach.

Results

Out of the 60 samples, 10 liver tissue samples showed positive results in FAdV PCR. Also, IBDV and CIAV were negative. IR/H3447-UTPCR/2020 was selected for sequencing. Sequence analysis of the loop region of the *hexon* gene revealed the highest identity of 94.58% of the virus detected in this study with FAdV serotype 7 detected in 2010 from Hungary (Fig. 2, Table 1). At the next level, IR/H3447-UTPCR/2020 Showed 93.96% homology with E7 FAdVs detected in China in 2018 and 2015 (Table 1).

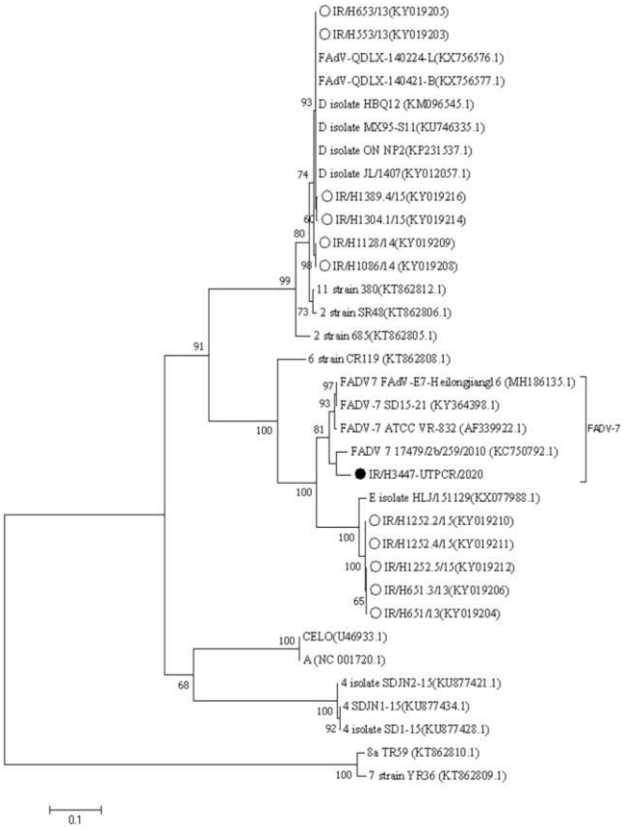

Fig. 2: Phylogenetic tree based on the loop region of the hexon gene of the fowl adenoviruses. Previously detected strains are shown with the white circles. The FAdV detected in this study is marked with a black circle

Table 1: Sequence identity matrix for IR/H3447-UTPCR/2020 and other related FAdVs, based on the loop region of the *hexon* gene

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	IR/H3447-UTPCR/2020													
2	FADV7_FAdV-E7-Heilongjiang16_(MH186135.1)	93.96												
3	FADV-7_SD15-21_(KY364398.1)	93.96	100.00											
4	FADV-7_ATCC_VR-832_(AF339922.1)	93.95	99.22	99.22										
5	FADV_7_17479/2b/259/2010_(KC750792.1)	94.58	95.61	95.61	95.20									
6	IR/H1389.4/15(KY019216)	58.44	57.43	57.43	58.12	55.88								
7	IR/H553/13(KY019203)	58.74	57.74	57.74	58.78	56.18	99.61							
8	D_isolate_HBQ12_(KM096545.1)	58.74	57.74	57.74	58.78	56.18	99.61	100.00						
9	E_isolate_HLJ/151129(KX077988.1)	85.00	86.46	86.46	85.97	83.77	55.46	55.75	55.75					
10	2_strain_SR48(KT862806.1)	60.09	60.14	60.14	61.14	58.66	96.45	96.85	96.85	57.90				
11	4_isolate_SDJN2-15(KU877421.1)	43.24	44.05	44.05	43.63	43.22	41.51	41.07	41.07	37.59	41.95			
12	6_strain_CR119_(KT862808.1)	82.82	83.61	83.61	84.34	83.78	62.69	63.31	63.31	77.75	65.54	43.94		
13	11_strain_380(KT862812.1)	59.08	59.13	59.13	60.15	57.62	97.05	97.45	97.45	57.21	99.03	41.12	64.60	

FAdVs: Fowl adenoviruses

Discussion

In Iran, FAdVs firstly detected belonged to D species (Hosseini and Morshed, 2012). Subsequently, an adenovirus-like IBH was reported in 2012 (Rahimi and Haghighi, 2015). Morshed *et al*. (2017) isolated FADVs from broiler and broiler breeder pullets with IBH. Partial *hexon* gene analysis revealed the presence of species D and E. They collected the samples from different provinces of Iran during 2013 and 2016. FAdV isolated from Golestan province belonged to species D, serotype 11 (Morshed *et al*., 2017). Another study was performed among broilers showing liver lesions and respiratory syndrome in northeast Iran. Sequence analysis of the L1 region of the *hexon* gene proved the existence of FAdVs from serotypes 2, 8b, and 11 (Nateghi *et al*., 2014). Khodakaram-Tafti *et al*. (2016) reported IBH based on histopathology examination among broilers of Fras province. Tabib Ghafari *et al*. (2018) molecularly detected FAdVs in broilers of the southwest of Iran. Analysis of the *hexon* gene revealed that the FAdVs belonged to serotype 11 (Tabib Ghafari *et al*., 2018). Recently, Hosseini *et al*. (2019) did full genome sequencing of an Iranian FAdV-11. According to the complete genome sequence analysis, UT-Kiaee had high homology with Chinese and Canadian FAdV. The partial sequence of the *hexon* gene revealed that UT-Kiaee shared 100% identity with previous Iranian FAdVs (Hosseini *et al*., 2019).

In 2020, broiler chickens in Gorgan slaughterhouse showed ecchymotic and petechial hemorrhages in thigh and breast muscles. Such hemorrhages in the meat are major quality defects affecting marketing chicken carcasses, and consumers do not usually admire them. We tried different strategies to differentiate other probable factors caused such hemorrhages. In the first step, to dispel possible mechanical problems in slaughtering procedure, or troubles originating from electrical stunning before slaughter, improvements were made in slaughtering methods, or even slaughtering was performed in some other slaughterhouses. However, hemorrhages were still being observed.

In the next step, we altered the source supplying chickens' feed to ensure that there is no toxins, mycotoxins, or fungi contaminating chicken rations. Vitamin-mineral supplementation was also increased in the broiler diet. Nevertheless, the problem was still present. Chickens did not represent clinical respiratory signs, were healthy, and the food conversion ratio was average. Finally, we looked for the viral pathogens that can induce hemorrhages in the chickens' tissues.

According to the results of this study, CIAV and IBDV were not detected, and tissue samples gave positive results in only FAdV PCR. Fowl adenovirus genome was detected in 70% of samples. One sample was selected for sequencing. Sequence analysis of the L1 region of the *hexon* gene clustered our virus with FAdVs from species E, serotype 7. IR/H3447-UTPCR/2020 showed the highest homology with a FAdV-E7 from Hungary. As shown in Fig. 2, previously detected FAdVs from Iran were from species D, and serotype 8 of species E. The virus detected in this study shared less than 85% identity with FAdVs from species E, serotype 8 detected during 2013 to 2016 in Iran. Fowl adenovirus from species E, serotype 7 has not yet been reported from Iran. There are also not many reports for the entire world. Fowl adenovirus was reported from India once in 1990 in broilers with IBH and another time in commercial broilers representing HPS and IBH (Singh *et al*., 2002; Mittal *et al*., 2014). Fowl adenovirus-7 was also reported in less than 3 weeks old broilers with IBH in Australia in 1991 (Erny *et al*., 1991). The next report was from layer chickens in Hungary in 2010 (Kaján *et al*., 2013).

According to the high number of FAdV infections in Iran in recent times, the effective monitoring of the virus at the slaughtering age of chicks from the whole country is an urgent mission. The viruses should be isolated, and their pathogenesis, especially the histopathological changes that they induce, should be studied. The complete genome sequence analysis may help understand the origin of the viruses and the pattern they spread.

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Conflict of interest

The authors declare that they have no conflict of

interest.

References

Chen, L; Yin, L; Zhou, Q; Peng, P; Du, Y; Liu, L; Zhang, Y; Xue, C and Cao, Y (2019). Epidemiological investigation of fowl adenovirus infections in poultry in China during 2015-2018. BMC Vet. Res., 15: 271.

Erny, K; Barr, D and Fahey, K (1991). Molecular characterization of highly virulent fowl adenoviruses associated with outbreaks of inclusion body hepatitis. Avian Pathol., 20: 597-606.

Hiremath, C; Jhala, M; Bhanderi, B and Joshi, CG (2013). Cloning and sequence analysis of *VP1*, *VP2* and *VP3* genes of Indian chicken anemia virus. Iran. J. Vet. Res., 14: 354-357.

Hosseini, H; Langeroudi, AG; FallahMehrabadi, MH; Kafi, ZZ; Dizaji, RE; Ghafouri, SA; Hamadan, AM; Aghaiyan, L and Hajizamani, N (2019). The fowl adenovirus (Fadv-11) outbreak in Iranian broiler chicken farms: The first full genome characterization and phylogenetic analysis. Comp. Immunol. Microbiol. Infect. Dis., 70: 101365.

Hosseini, H and Morshed, R (2012). Molecular identification of fowl adenovirus associated with inclusion body hepatitis in Iran. Iran. J. Virol., 6: 7-12.

Kaján, GL; Affranio, I; Bistyák, AT; Kecskeméti, S and Benkő, M (2019). An emerging new fowl adenovirus genotype. Heliyon. 5: e01732.

Kaján, GL; Kecskeméti, S; Harrach, B and Benkő, M (2013). Molecular typing of fowl adenoviruses, isolated in Hungary recently, reveals high diversity. Vet. Microbiol., 167: 357-363.

Kefford, B; Borland, R; Slattery, J and Grix, D (1980). Serological identification of avian adenoviruses isolated from cases of inclusion body hepatitis in Victoria, Australia. Avian Dis., 24: 998-1006.

Khodakaram-Tafti, A; Asasi, K and Namazi, F (2016). Clinicopathological characteristics of acute inclusion body hepatitis outbreak in broiler chickens in Iran. Bulg. J. Vet. Med., 19: 163-168.

Mittal, D; Jindal, N; Tiwari, AK and Khokhar, RS (2014). Characterization of fowl adenoviruses associated with hydropericardium syndrome and inclusion body hepatitis in broiler chickens. Virus Dis., 25: 114-119.

Morshed, R; Hosseini, H; Langeroudi, AG; Fard, MHB and Charkhkar, S (2017). Fowl adenoviruses D and E cause inclusion body hepatitis outbreaks in broiler and broiler breeder pullet flocks. Avian Dis., 61: 205-210.

Nateghi, E; Razmyar, J and Bassami, MR (2014). Molecular characterization of avian adenoviruses in Iranian broiler flocks. Iran. J. Vet. Res., 15: 164-167.

Rahimi, M and Haghighi, ZMS (2015). Adenovirus-like inclusion body hepatitis in a flock of broiler chickens in Kermanshah province, Iran. Vet. Res. Forum., 6: 95-98.

Razmyar, J and Peighambari, SM (2009). Isolation and characterization of a very virulent infectious bursal disease virus from turkey. Acta Virol., 53: 271-276.

Ren, G; Wang, H; Huang, M; Yan, Y; Liu, F and Chen, R (2019). Transcriptome analysis of fowl adenovirus serotype 4 infection in chickens. Virus Genes., 55: 619-629.

Sapats, SI and Ignjatovic, J (2002). Restriction fragment length polymorphism analysis of the *VP2* gene of Australian strains of infectious bursal disease virus. Avian Pathol., 31: 559-566.

Singh, A; Oberoi, M; Grewal, G; Hafez, H and Hess, M (2002). The use of PCR combined with restriction enzyme analysis to characterize fowl adenovirus field isolates from northern India. Vet. Res. Commun., 26: 577-585.

Steer, PA; Kirkpatrick, NC; O'Rourke, D and Noormohammadi, AH (2009). Classification of fowl adenovirus serotypes by use of high-resolution melting-curve analysis of the hexon gene region. J. Clin. Microbiol., 47: 311-321.

Tabib Ghafari, P; Boroomand, Z; Rezaie, A; Mayahi, M and Eftekharian, S (2018). Detection and identification of avian adenovirus in broiler chickens suspected of inclusion body hepatitis in Khuzestan, Iran during 2015-2016. Iran. J. Vet. Res., 9: 41-45.