Iranian Journal of Virology 2022;16 (1):54-58
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Original Article

Molecular Identification and Phylogenetic Analysis of a Novel Strain of Duck Picornavirus (Aalivirus) in Iran: The First Report

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Abstract

We detected a novel strain of picornavirus from dead backyard ducks from Gilan province, In Iran. This new isolated was demonstrated a genome like picornavirus design, specifically Aalivirus (Avihepatovirus/Avisivirus-like virus). Usually, Aalivirus were observed in domestic ducks, turkey and chickens. Ten pooled feces samples were collected from samples. We implemented partial genome sequencing of VP1 gene. We nominated the new strain as duck Aalivirus isolate UT-Ebrahimi. Based on our investigation with phylogenetic tree, we considered this strain is a member of the Aalivirus genus of picornavirus. In this study, we demonstrated that the UT-Ebrahimi is 96.23 % similar to Pacific_black_duck_megrivirus_(MK204391.1).

Keywords: DHAV, picornavirus, duck, detection, Iran

Introduction

Duck hepatitis virus (DHV) is one of the significant economic importance in duck growing farms; which can lead to high mortality and significant economic losses. The DHV infection usually prevalence by most pathogenic and widespread type, DHV-1 (1).

DHV-1 can cause high mortality and rapid distribution in young ducklings (1, 2). Based on the International Committee on Taxonomy of Viruses (ICTV), the DHV-1 renamed to duck hepatitis A virus type 1 (DHAV-1) and classified in the *Avihepatovirus* genus of Picornaviridae family (3).

The viruses of Picornaviridae family are small non-enveloped viruses with an icosahedral capsid and positive-sense, single-stranded, and polyadenylated RNA genomes with a poly (A) tail at the 3′ end (1). There is a flanke of 5′ and 3′ untranslated regions (UTRs) in the open reading frame (ORF) of the typical picornavirus, DHAV-1 genome. The ORF can be decoded a large polyprotein, which produced 12 mature products including structural (VP0, VP3, and VP1) and nonstructural (2A1, 2A2, 2A3, 2B, 2C, 3A, 3B,3C, and 3D) proteins (1, 2, 4). VP0, VP1, and VP3 genes encode capsid proteins (viral antigen epitopes), which lead to specific antigenicity (1, 5).

Results of sequence analysis have demonstrated that mutations mainly were occurred in the gene encoding VP1 (1, 5-7). VP1 is one of the structural proteins , that probably plays a critical role in receptor binding, virulence, immunogenicity, and protection (1, 5).

The Piconaviridae family subdivided into 17 genus, including *Aphthovirus*, *Aquamavirus*, *Avihepatovirus*, *Cardiovirus*, *Cosavirus*, *Dicipivirus*, *Enterovirus*, *Erbovirus*, *Hepatovirus*, *Kobuvirus*, *Megrivirus*, *Parechovirus*, *Salivirus*, *Sapelovirus*, *Senecavirus*, *Teschovirus* and *Tremovirus*. Furthermore, nine new genus have been discoverded namely ‘Avisivirus’, ‘Gallivirus’, ‘Hunnivirus’, ‘Mischivirus’, ‘Mosavirus’, ‘Oscivirus’, ‘Pasivirus’, ‘Passerivirus’ and ‘Rosavirus’ (http://www.picornastudygroup.com/proposals/2013/psg_proposals_2013.htm).

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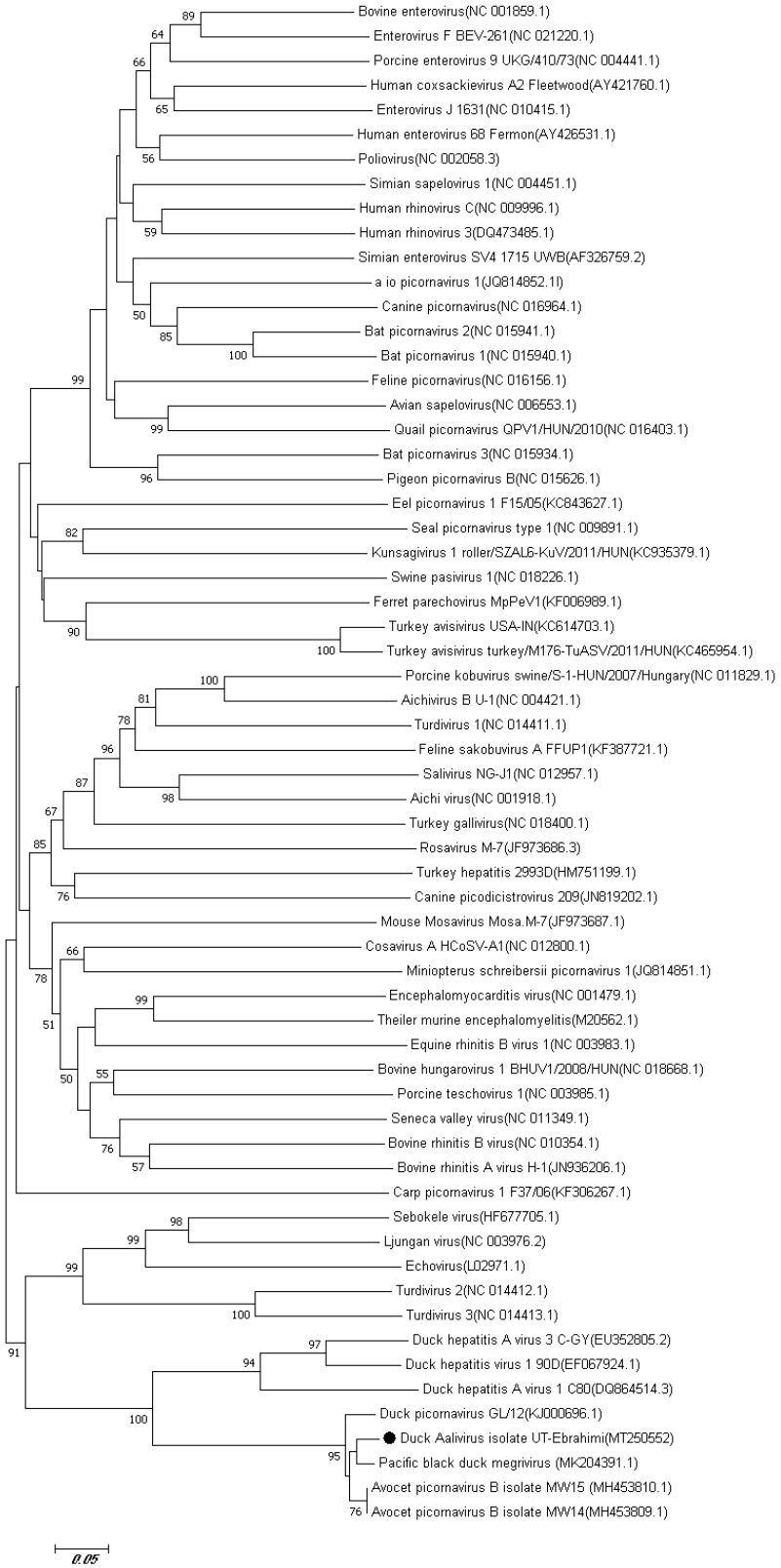


Fig. 1. Phylogenic tree drawn, on the basis of the VP1 partial gene sequences of new strain of picornavirus, the UT-Ebrahimi, using the neighbor-joining method 1000 bootstrap replications.

Therefore, it may be a number of picornaviruses that don't be classified yet (1). Three novel duck picornaviruses have been found in domestic ducks including *Sapelovirus*, *Megrivirus*, and new *Aalivirus* genus (2). The Avihepatovirus, Avisivirus and Aalivirus have several viruses in domestic ducks, turkey, and chickens; such as duck Hepatitis A viruses (DHAV) of genus Avihepatovirus, Aalivirus A1 in Aalivirus genus in ducks and Avisivirus A1 in Avisivirus genus in turkeys (8). DHV-1 observed in young White Pekin ducks on Long Island in 1945, for the first time (1, 9).

Afterward, the DHV-1 has been spread worldwide in duckraising areas (10). DHAV-1 isolation for the first time was in chicken embryos in 1950 (1, 11).

The complete genome analysis was implementted in 2006; therefore, it is categorized in the *Avihepatovirus* genus of the picornaviride family (12, 13). Here, we decided to report a novel strain of picornavirus from Iranian backyard ducks.

Methods

In the present study, ten intestinal feces samples were collected from dead backyard poultry (ducks), in Gilan province, Iran, 2018. The samples were pooled and were stored at -70 °C. Gilan Province is one of the prominent wetlands of Iran, which converts Iran to a critical ecological site for wild migratory birds in the Central Asia pathways. Total RNA extraction was stored at -70°C until used (14).

Every 10 samples from each farm were homogenized, and the total RNA of samples was extracted by using the TRIzol Plus RNA Purification Kit (Thermo Fisher Scientific, USA). It was performed following the manufacturer's instructions.

After extraction, 30 µl of each of the samples from 10 farms were pooled. It was sent to Beijing Genomics Institute (BGI, China) and sequenced using the Illumina HiSeq 4000 platform in two separate runs, generating paired-end 150 bp reads. Next generation sequencing of pooled RNA extraction samples was performed by Illumine Hiseq4000 (BGI, China). The high throughput sequencing reads accurately assembled whole known viral genomes by web-based Genome Detective briefly described in the following (15).

We performed partial genome sequencing of VP1 gene. Sequence results were assessed from the perspective of the reads quality and bioinformatics analysis. The quality of the reads was evaluated using FastQC (16). The low-quality reads were filtered and adapters trimmed with Trimmomatic (17). The viral reads were identified using the DIAMOND method, and non-viral reads were removed (18). The viral reads de novo assembled using metaSPAdes, and viral contigs ascertained (15, 19). Identified viral taxa represented by Krona (20).

Results

The VP1 nucleotid sequence was deposited in the GenBank database (NCBI) under accession number of MT250552. We nominated the new strain as duck Aalivirus isolate UT-Ebrahimi.

Based on the phylogeny tree, our isolated in contrast to the other picornaviride family viruses, the UT-Ebrahimi is 91.21 % similar to duck picornavirus GL/12(KJ000696.1) and 96.23% to Pacific_black_duck_megrivirus_ (MK204391.1) (Fig. 1). The UT-Ebrahimi is 95.92 % similar to Avocet_picornavirus_B_ isolate_MW15_(MH453810.1) and also 95.92 % to Avocet_picornavirus_B_isolate_MW14 (MH453809.1) (Table .1).

Our isolated was classified into the Aalivirus genus. Our results demonstrated that the new Iranian strain of DHV, the UT-Ebrahimi, is a member of Aalivirus genus.

Discussion

DHAV was subdevided to three serotypes, including DHAV-1, DHAV-2, and DHAV-3. There is no cross antigenicity among them (21). Earlier DHV-1 was classified as an enterovirus.

Table 1. comprasion of the partial VP1 gene of the UT-Ebrahimi with other strains of DHV						
		1	2	3	4	5
1	Duck_Aalivirus_isolate_UT-Ebrahimi (MT250552)					
2	Pacific_black_duck_megrivirus_(MK204391.1)	96.23				
3	Avocet_picornavirus_B_isolate_MW15_(MH453810.1)	95.92	95.92			
4	Avocet_picornavirus_B_isolate_MW14(MH453809.1)	95.92	95.92	100.00		
5	Duck_picornavirus_GL/12(KJ000696.1)	91.21	96.23	95.92	95.92	

In 2006, some studies implemented complete nucleotide sequences of DHV-1 to accurate recogniz. One of the studies has been determined the two strains DRL-62 and R85952 of DHV-1. They indicated that the two strains are more similar to Parechovirus genus, but was different from members of this genus, based on phylogeny analysis (12). Also complete genome sequence of a DHV-1 strain C80 in China demonstrated that the DHV-1 is one of the picornaviride family and a separate and similar genus to parechovirus (22).

Tseng, C.-H. et al. (2007) implemented an investigation on the occurrence of DHV in many vaccinated ducklings in Taiwan, with DHV-1 serotype.

They demonstrated the presence of a novel DHV, N-DHV, which different from DHV-1 antigenically; They isolated a new serotype of the DHV of picornavirus. They indicated that despite the similarity of N-DHV to DHV-1, they belonged to two different DHV serotypes (13).

In a study, Wang, X., et al. (2014) isolated a novel virus from ducks in a commercial Pekin duck flock in China. They indicated that the new virus was similar to the picornavirus. The new virus in their results of amino acid identities was most closely related to DHAV.

Based on the phylogenic analysis, they expressed, the virus could be a member of the picornavirus genus as a novel virus, which they nominated as Aalivirus (2). Some researchers collected duck livers samples from the south of china during 2006-2007, and determinated the complete genome sequence of an isolate of DHV-I. They also sequenced the VP1 gene of nine southeasts China field isolated viruses and three strains of attenuated DHV-I vaccine. The phylogeny analysis in this study indicated, the attenuated and tissue-adapted isolates were significantly different from the field isolates and have been clustered in separate genotypes.

The authors suggested that some of these differences may be consequences of mutations (5). Boros, Á., et al. were performed an investigation in broiler chicken for identification of a novel picornavirus, Orivirus A1. They demonstrated that the Orivirus A1 was correlated to the members of Avihepatovirus and Aalivirus (8).

Wei, C.-y., et al. (2012) reported gene association among DHAV-1 and DHAV-3, for the first time. They implemented the complete genomic sequence of a new DHAV of mixed infection of DHAV-1 and DHAV-3 in ducklings in Southern China. The whole sequences indicated high homology among them (7). A study was performed in the Hubei province of China; Researchers isolated a new strain of DHV from infected ducklings with clinical symptoms. The comparative complete genome analysis of new strain (JX) with other available strains of GenBank demonstrated that new strain was 94-99% similar to the others, based on nucleotide level. The differences were occured by mutations of nucleotide and amino acid, mainly in VP1 genes. They intimated that the VP1 gene possibly was the most virulent reason in DHV-1 (23).

Conclusion

In conclusion, we discovered a novel strain of DHAV in backyard ducks in Gilan Province, in Iran. Additionally, we implemented partial sequencing of the genome and nominated the picornavirus to UT-Ebrahimi. This novel starin classified to the Aalivirus genus of picornavirus.

Acknowledgment

The authors would like to thank experts of the Ghalyanchi lab at the University of Tehran for their extensive technical supports.

Conflict of interest

No conflict of interest is declared.

Funding

None.

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