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Short Communication

Characterization of Iranian Avian Metapneumovirus Based on Fusion Gene (F)

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Abstract

Avian metapneumovirus (aMPV) causes one of the most prevalent diseases of poultry mainly in combination with other pathogens, and it is increasing among chickens. In the present study, the detection and characterisation of an aMPV subtype B strain circulating in broiler flocks based on fusion (F) gene. In phylogenetic analysis, the isolates are located in B subtype cluster and near aMPV strains from Russia (99.58%). It is the first molecular characterisation based on F gene of aMPV in Iran. It is also concluded that more work is required to isolate and characterize aMPV in different geographical regions of Iran and several species.

Keywords: Avian metapneumovirus; Detection; Characterization; Fusion; Phylogenetic Study.

Introduction

Avian metapneumovirus (aMPV) is an economically important infection of chicken and turkey causing both respiratory and reproductive symptoms which can be mainly severe in the presence of complicating pathogens. aMPV belongs to the Pneumovirinae family, Metapneumovirus genus. It is a single-stranded, negative-sense RNA (14 kb) virus containing eight genes organized in the following order: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), matrix 2 (M2), small hydrophobic (SH), attachment glycoprotein (G), and large polymerase (L) gene. The putative gene order of APV(N-P-M-F-M2-SH-G-L) is different from its mammalian counterparts (NS1-NS2-N-P-M-SHG-F-M2-L), wherein the SH and G genes are located 5% to the M2 gene(1). Currently, four subtypes (A, B, C, and D) are defined based on genomic difference analysis, and they have been identified in several avian species. The G proteins share approximately 99% identity among viruses in the same group.

However, they are only38% similar between viruses from the two aMPV subtypes. This correlates with earlier data demonstrating that various aMPV isolates were antigenically similar, but could be separated serologically into two distinct groups.

This relationship was further confirmed by sequence analysis of the F protein gene and the more conserved M gene.

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Differences the subtype A and B display a worldwide distribution while the subtype C has been detected in the United States, France, Korea, and more recently in China while subtype D has only been detected once, in France (2). In Iran, subtype B has been isolated from commercial broiler and breeder flocks.

Despite the use of different vaccination programs, including live and inactivated aMPV A and B vaccines, respiratory disease, decreased egg production, and high serocon-version have been observed in vaccinated flocks (3, 4). A diagnosis may be made by virus isolation, serology, or reverse transcri-ption-polymerase chain reaction (RT-PCR). Virus isolation procedure of aMPV is hard and time-consuming. Current serology tests include immunofluorescence (FA), virus neutralisation (VN), or enzyme-linked immunosorbent assay (ELISA) .

The RT-PCR can be used for differentiation and detection of aMPV. Tracheal swabs and turbinates are considered to be the most appropriate sources of aMPV (4). In Iran, subtype B have been isolated from commercial broiler and breeder flocks. Despite the use of different vaccination programs, including live and inactivated aMPV A and B vaccines, respiratory disease, decreased egg production, and high serocon-version have been observed in vaccinated flocks (3, 4).

Material and Methods

Total RNA was extracted from tissue with the RNeasy mini kit (Qiagen, Germany) according to manufacturer′s instructions and then stored at -70. The cDNA was synthesized using RevertAid first strand cDNA synthesis Kit (Thermo Fisher Scientific, Canada).

Polymerase chain reaction (PCR) was performed using Fusion gene primers, which were designed to amplify approximately 390 bp fragment of F gene. PCR was performed in 20 µl volumes in a mixture containing 2µl of distilled water, 2 µl of primers (10 µM) from F1 , 3 µl of cDNA, and 13 µl of Sinaclon 2X PCR master mix (Sinaclon, Iran). The amplification reaction was performed for an initial denaturation period at 94°C for 2 min and 35 cycles with denaturation at 94°C for 15 s, annealing at 58°C for 30 s, and polymerization at 72°C for 30 s. Final extension was performed at 72°C for 10 min.

The AccuPrep® PCR purification Kit (Bioneer Co., Korea) was used for purification of the PCR products (315 bp). Sequencing was performed with the primers (both directions) (Bioneer Co., Korea). Chromatograms were evaluated with CromasPro (CromasPro Version 1.5).

All reference sequences used in this study were sourced from the NCBI GenBank database. Sequence homology analysis was performed using MEGA7.0.

Phylogenetic trees were constructed using MEGA7.0 with the neighbour-joining algorithm (bootstrap values of 1000) with the Kimura2 parameter model (5).

Results

After receiving sequences and editing them for confirmation of obtained band, we did BLAST and results of the BLAST confirmed the presence of aMPV RNA in the samples. Bioinformatics analysis and a phylogenetic tree based on partial nucleotide sequences of the F gene shows that the detected aMPVs were classified in the subtype B (Figure 1). Detected strains were established phylogenetically in a separate branch. Similarity among Iranian strains was 99.58% -100 % (Table 1).

The similarity of Iranian aMPV to type A, C and D were 77.07%,76.4% and 68.41%. The Iranian aMPV strain has the highest similarity (99.58%) to Russian type B isolates. It should be noted that negative extraction control and PCR reaction negative control were both negative that represents the accuracy of the work.

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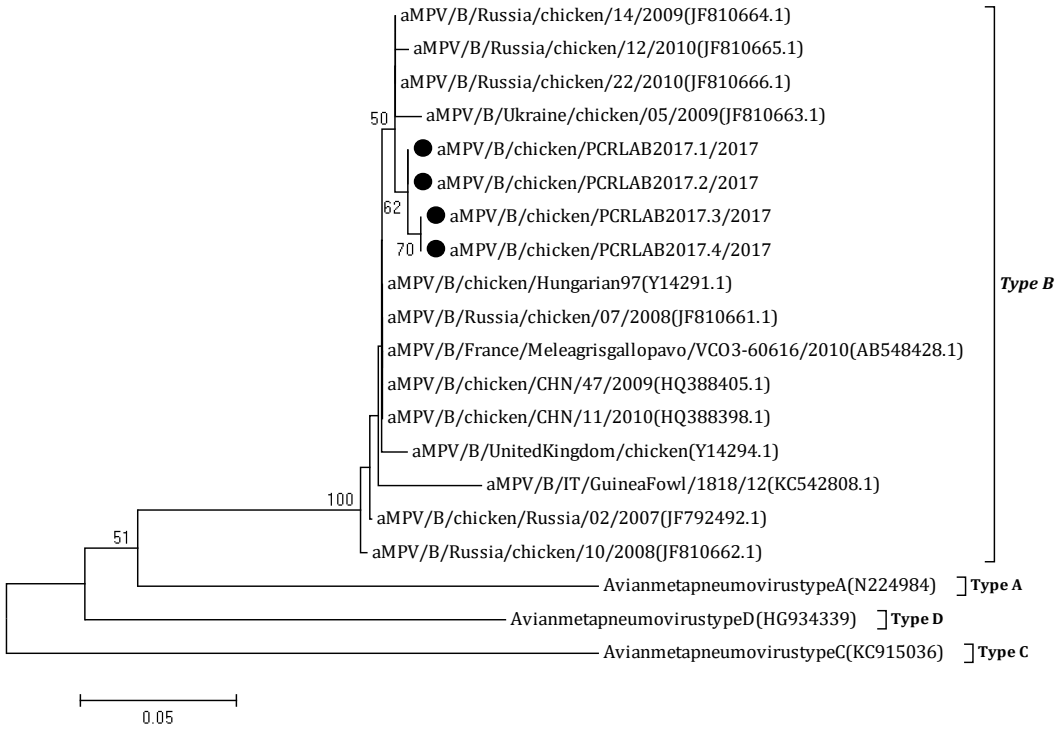


Fig. 1. Phylogenetic tree constructed with F gene sequences of aMPV reference strains and field isolates. The analysis was done with the Neighbor-Joining method, Kimura 2 parameters (MEGA 7). Numbers at nodes correspond to bootstrap values (1000 replications). Iranian strains are marked with a black square. The Iranian strains were compared to all aMPV subtypes A, B, C and D N gene sequences available in Genbank.

Table 1: Percent identity of partial nucleotide sequences of the F gene of aMPV B detected in commercial broiler chickens to those of aMPV reference strains

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	aMPV/B/chicken/Hungarian97(Y14291.1)	100																			
2	aMPV/B/UnitedKingdom/chicken(Y14294.1)	99.17	100																		
3	aMPV/B/Russia/chicken/10/2008(JF810662.1)	99.16	98.3	100																	
4	aMPV/B/Russia/chicken/07/2008(JF810661.1)	100	99.2	99.2	100																
5	aMPV/B/chicken/Russia/02/2007(JF792492.1)	99.58	98.7	99.6	99.58	100															
6	aMPV/B/Russia/chicken/22/2010(JF810666.1)	99.58	98.7	98.7	99.58	99.2	100														
7	aMPV/B/Russia/chicken/14/2009(JF810664.1)	99.58	98.7	98.7	99.58	99.2	100	100													
8	aMPV/B/Ukraine/chicken/05/2009(JF810663.1)	98.74	97.9	97.9	98.74	98.3	99.16	99.2	100												
9	aMPV/B/Russia/chicken/12/2010(JF810665.1)	99.16	98.3	98.3	99.16	98.7	99.58	99.6	98.7	100											
10	aMPV/B/IT/GuineaFowl/1818/12(KC542808.1)	96.59	95.7	95.7	96.59	96.1	96.15	96.1	95.2	95.7	100										
11	aMPV/B/France/Meleagrisgallopavo/VCO3-60616/2010(AB548428.1)	100	99.2	99.2	100	99.6	99.58	99.6	98.7	99.2	96.6	100									
12	aMPV/B/chicken/CHN/47/2009(HQ388405.1)	100	99.2	99.2	100	99.6	99.58	99.6	98.7	99.2	96.6	100	100								
13	aMPV/B/chicken/CHN/11/2010(HQ388398.1)	100	99.2	99.2	100	99.6	99.58	99.6	98.7	99.2	96.6	100	100	100							
14	aMPV/B/chicken/PCRLAB2017.1/2017	99.17	98.3	98.3	99.17	98.7	99.58	99.6	98.7	99.2	95.7	99.17	99.17	99.2	100						
15	aMPV/B/chicken/PCRLAB2017.2/2017	99.17	98.3	98.3	99.17	98.7	99.58	99.6	98.7	99.2	95.7	99.17	99.17	99.2	100	100					
16	aMPV/B/chicken/PCRLAB2017.3/2017	98.74	97.9	97.9	98.74	98.3	99.17	99.2	98.3	98.7	95.3	98.74	98.74	98.7	99.58	99.6	100				
17	aMPV/B/chicken/PCRLAB2017.4/2017	98.74	97.9	97.9	98.74	98.3	99.17	99.2	98.3	98.7	95.3	98.74	98.74	98.7	99.58	99.6	100	100			
18	AvianmetapneumovirustypeC(KC915036)	68.38	67.8	69	68.38	68.4	68.38	68.4	69	67.7	65.1	68.38	68.38	68.4	68.41	68.4	69.06	69.1	100		
19	AvianmetapneumovirustypeA(N224984)	77.12	77.2	78.3	77.12	77.7	76.52	76.5	75.9	75.9	76	77.12	77.12	77.1	77.07	77.1	76.46	76.5	65.1	100	
20	AvianmetapneumovirustypeD(HG934339)	77.07	76.5	77.1	77.07	77.7	76.46	76.5	77.1	77.1	73.5	77.07	77.07	77.1	76.4	76.4	76.4	76.4	65	67.03	100

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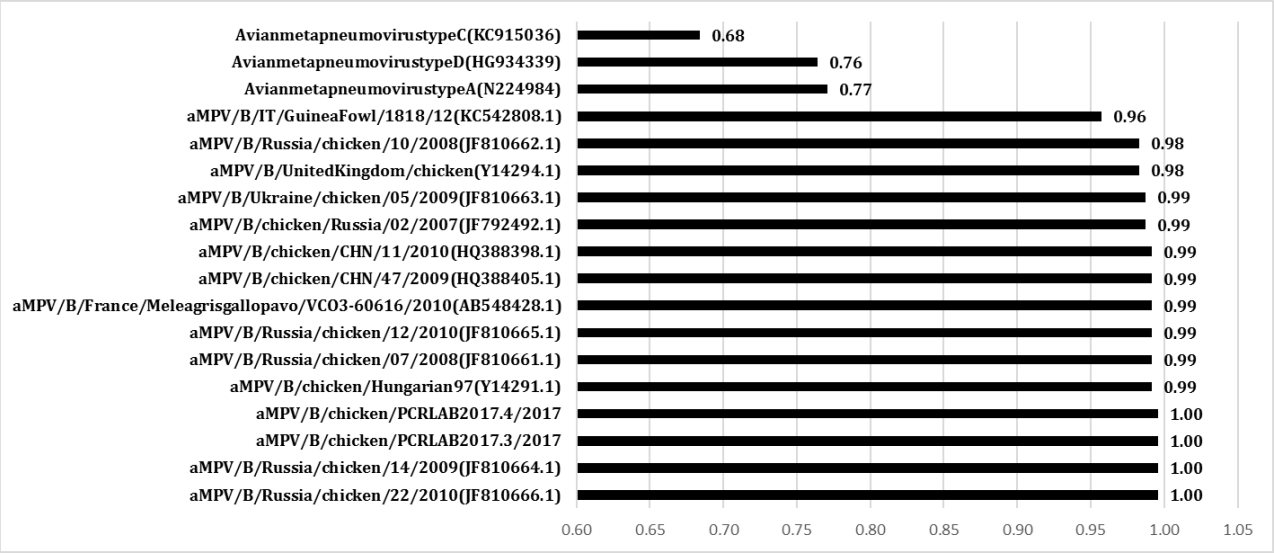


Fig. 2. Comparison of percent identity of Fusion gene of some APVs to aMPV/B/chicken/PCRLAB2017.1/ 2017.

Discussion

Avian metapneumoviruses (known as avian pneumoviruses or aMPV) cause respiratory diseases and egg drops in species such as chicken, turkey, Muscovy or Pekin duck (6). First reported in the late 1970s in South Africa, and subsequently, in France and the UK, aMPVs have now been described worldwide (7). Clinical signs are not pathognomonic for diagnosis of aMPV. A diagnosis may be made by virus isolation, PCR, or serology. The first detection of aMPV in broiler flocks published in 2010 and further studies confirmed the presence of group B of aMPV in Iran flocks. Till today, both killed and live aMPV vaccines are used in the broiler, parent and layer flocks (3, 8). Our study can characterize the aMPV based on F gene for the first time in Iran. No sequences of aMPV base on fusion gene were available before the study. In the USA, the F gene among US aMPV isolates shared 98% nucleotide sequence identity, 9 conserved substitutions were detected in the predicted amino acid (AA) sequence. The predicted AA sequence of the US aMPV isolate's F protein had 72% sequence identity to subtype A and 71% identity to subtype B. This compares with 83% sequence identity between the APV subtype A and B predicted AA sequences of the F protein. The US isolates were phylogenetically distinguishable from their European isolates based on F gene predicted amino acid or nucleotide sequences. Lack of sequence heterogeneity among US aMPV isolates shows these viruses have maintained a relatively stable since the first outbreak of TRT (1).

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