Prevalence of avian metapneumovirus subtype B in live bird market in Gilan province, Iran

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Article Info	Abstract
	Avian metapneumovirus (aMPV), also known as avian pneumovirus or turkey rhinotracheitis virus, is the causative agent of turkey rhinotracheitis and swollen head syndrome in chickens. Four aMPV subgroups (A-D) have been reported previously based on their genetic and antigenic differences. Evidence suggests that the live bird markets (LBMs) play an important role in the epidemiology of the avian viral diseases. A total number of 450 oropharyngeal samples from eight different species of birds (migratory and local) were collected from LBMs of Gilan province, Iran, from October to December 2016. The presence of aMPV was determined by reverse transcription polymerase chain reaction (RT-PCR) based on nucleoprotein gene. The aMPV was detected in 30.60% of the examined birds including chickens (37.00%), turkey (33.00%), Eurasian teal (25.00%), common blackbird (33.00%), and Eurasian woodcock (25.00%). Bioinformatics analysis and a phylogenetic tree based on partial nucleotide sequences of the N gene showed that the detected aMPVs were belonged to subtype B. This is the first report of aMPV in non-commercial birds in Iran. Knowledge of the frequency and types of infected birds with pneumoviruses allow a better understanding of the epidemiology of aMPV in Iran. © 2018 Urmia University. All rights reserved.

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شیوع متاپنوموویروس پرندگان تیپ B در بازار پرندگان زنده در استان گیلان، ایران

چکیده

متاپنوموویروس پرندگان همچنین تحت‌عنوان پنوموویروس پرندگان یا ویروس رینوتراکئیت بوقلمون شناخته می‌شود و عامل مسبب رینوتراکئیت بوقلمون و سندرم سر متورم در جوجه‌ها می‌باشد. براساس تفاوت‌های ژنتیکی و آنتی‌ژنی چهار زیر گروه (A-D) از aMPV گزارش شده‌است. بازارهای فروش پرنده زنده نقش مهمی در همه‌گیری بیماری‌های ویروسی پرندگان ایفا می‌کنند. تعداد ۴۵۰ نمونه دهانی حلقی از ۱۰ گونه مختلف پرندگان وحشی (مهاجر و محلی) در استان گیلان از مهر تا آذر سال ۱۳۹۵ در بازار پرندگان جمع‌آوری گردید. حضور پنوموویروس توسط واکنش زنجیره‌ای پلیمراز براساس ژن نوکلئوپروتئین ردیابی گردید. پنومو ویروس در ۳۰/۶۰ درصد پرندگان مورد آزمایش از جمله مرغ (۳۷/۰۰ درصد)، بوقلمون (۳۳/۰۰ درصد)، خوتکا (۲۵/۰۰ درصد)، توکای سیاه (۳۳/۰۰ درصد) خروس جنگلی اروپایی-آسیایی (۲۵/۰۰درصد) شناسایی گردید. ارزیابی بیوانفورماتیکی و درخت فیلوژنیک براساس توالی‌های نوکلئوتیدی ژن نوکلئوکپسید نشان داد که پنوموویروس‌های جدا شده متعلق به زیر گروه B بودند. این اولین گزارش از پنوموویروس در پرندگان غیر تجاری در ایران است. اطلاعات در زمینه فراوانی و نوع پرنده عفونی باعث درک بهتر ما از اپیدمیولوژی این بیماری در کشور خواهد شد.

واژه های کلیدی: ایران، بازار پرندگان زنده، پنوموویروس پرندگان، مطالعه شجره‌شناسی

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Introduction

Avian metapneumovirus (aMPV), a member of family Paramyxoviridae, subfamily Pneumovirinae, genus Metapneumovirus is a single-stranded, negative-sense RNA virus that causes rhinotracheitis and/or swollen head syndrome in chickens.[1] The aMPV genome consists of approximately 13 kb of a non-segmented, linear, negative-sense strand RNA, which encodes eight genes: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), second matrix (M2), small hydrophobic (SH), surface glycoprotein (G), and RNA dependent RNA polymerase (L) in the gene order 5'-N-P-M-F-M2-SH-G-L-3'.[2] The aMPV causes turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) of chickens, which is usually accompanied by secondary bacterial infections that increase mortality in the affected birds.[3] The aMPV was first reported in South Africa in1978 and was later detected in France, the UK, Israel, Asia, Brazil and the USA. The aMPV is widely distributed throughout the world, except in Australia.[4] There are four distinct aMPV subtypes; A, B, C and D. Subtypes A, and B are widespread throughout Asia, Europe, Africa and South America. Reports of infections by subtypes C and D are infrequent. To date, subtype C has been reported from France, Korea, and the USA, and subtype D so far only has been detected in France.[4] Diagnosis of aMPV infections may be made by serology, reverse transcription-polymerase chain reaction (RT-PCR) or virus isolation. Virus isolation of aMPV is time-consuming and difficult. Current serology tests include enzyme-linked immunosorbent assay (ELISA), and virus neutralization (VN) and immunofluorescence (IF). The RT-PCR can be used for detection and differentiation of aMPV. Tracheal swabs are considered to be the most appropriate sources of aMPV.[5] In Iran, aMPV subtype B have been isolated from commercial broiler and breeder flocks. Despite the employment of different vaccination programs, including live and inactivated aMPV-A and aMPV-B vaccines, respiratory disease, decreased egg production, and high seroconversion have been observed in vaccinated flocks.[3,5] The purpose of this study was to detect of aMPV in in live bird market in Gilan province, north of Iran.

Materials and Methods

Oropharyngeal swabs (n = 345) from chickens (*Gallus gallus*), turkey (*Meleagris gallopavo*), Eurasian teal (*Anas Crecca*), common blackbird (*Turdus merula*), and Eurasian woodcock (*Scolopax rusticola*) from live bird market of Gilan province, north of Iran, were collected. RNA was extracted from swab samples using high pure RNA extraction kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer instructions. deionized water was used as the negative control during RNA extraction and PCR procedure. The extracted RNA was stored in –70 °C until use. The amplification assay was standardized using a one-step RT-PCR kit (Qiagen, Hilden, Germany). The RT-PCR assay was performed in a final volume of 25 µL containing 5 µL 5x Qiagen one-step RT-PCR Buffer, 1 µL of enzyme mix, 0.60 µM of each primer, 1 µL dNTP mix (containing 10 mM of each dNTP) and 2.5 µL RNA. The primers including ND 5' AGCAGGATGGAGA GCCTCTTTG 3' and NX 5' CATGGCCCAACATTATGTT 3' targeted the N gene.[6] In one-step the RT-PCR procedure, the RNA was transcribed at 50 °C for 30 min. This was followed by one cycle of 94 °C for 15 min for activation of the Taq DNA polymerase and inactivation of reverse transcriptase. The amplification was performed in 40 cycles of denaturation at 94°C for 25 sec, annealing at 50 °C for 30 sec and extension at 72 °C for 45 sec. Amplified PCR products (115 bp) were visualized on a 2.00% agarose gel. The PCR products were purified using AccuPrep® PCR purification kit (Bioneer Co., Daejeon, Korea). Sequencing was performed using the same primers used in the PCR in both directions. Chromatograms were evaluated using CromasPro (version 1.5; Technelysium, Tewantin, Australia). The N gene sequences were aligned with the corresponding region of the N gene sequences from GenBank. The genetic distances of the aligned sequences were calculated using the Kimura-two parameter model in the MEGA software (version 7.0; Biodesign Institute, Tempe, USA). The phylogenetic tree was constructed using the Neighbor-Joining algorithm in the program using a consensus of 1000 bootstrap replicates.[7]

Results

The aMPV was detected in 30.60% of the examined birds. The frequency of aMPV infection in different bird species was 37.00% in chickens (*Gallus gallus*), 33.00% in turkey (*Meleagris gallopavo*), 25.00% in Eurasian teal (*Anas Crecca*), 33.00% in Common blackbird (*Turdus merula*), and 25.00% in Eurasian woodcock (*Scolopax rusticola*). Blast search, bioinformatics analysis and a phylogenetic tree based on partial nucleotide sequences of the *N gene* showed that the detected aMPVs belonged to subtype B of aMPV (Fig. 1). The aMPVs detected in the present study showed 78.40 to 80.20% similarity with aMPV Nemovac vaccine strain (Merial, Lyon, France). Detected aMPVs were established phylogenetically in a separated branch. The similarity among aMPVs was 97.28% to 100% (Table 1).

Discussion

Wild and domestic birds from LBM have been considered as important reservoirs for avian viruses such as avian influenza and Newcastle disease.[8] The understanding of the epizootiology of aMPV is very
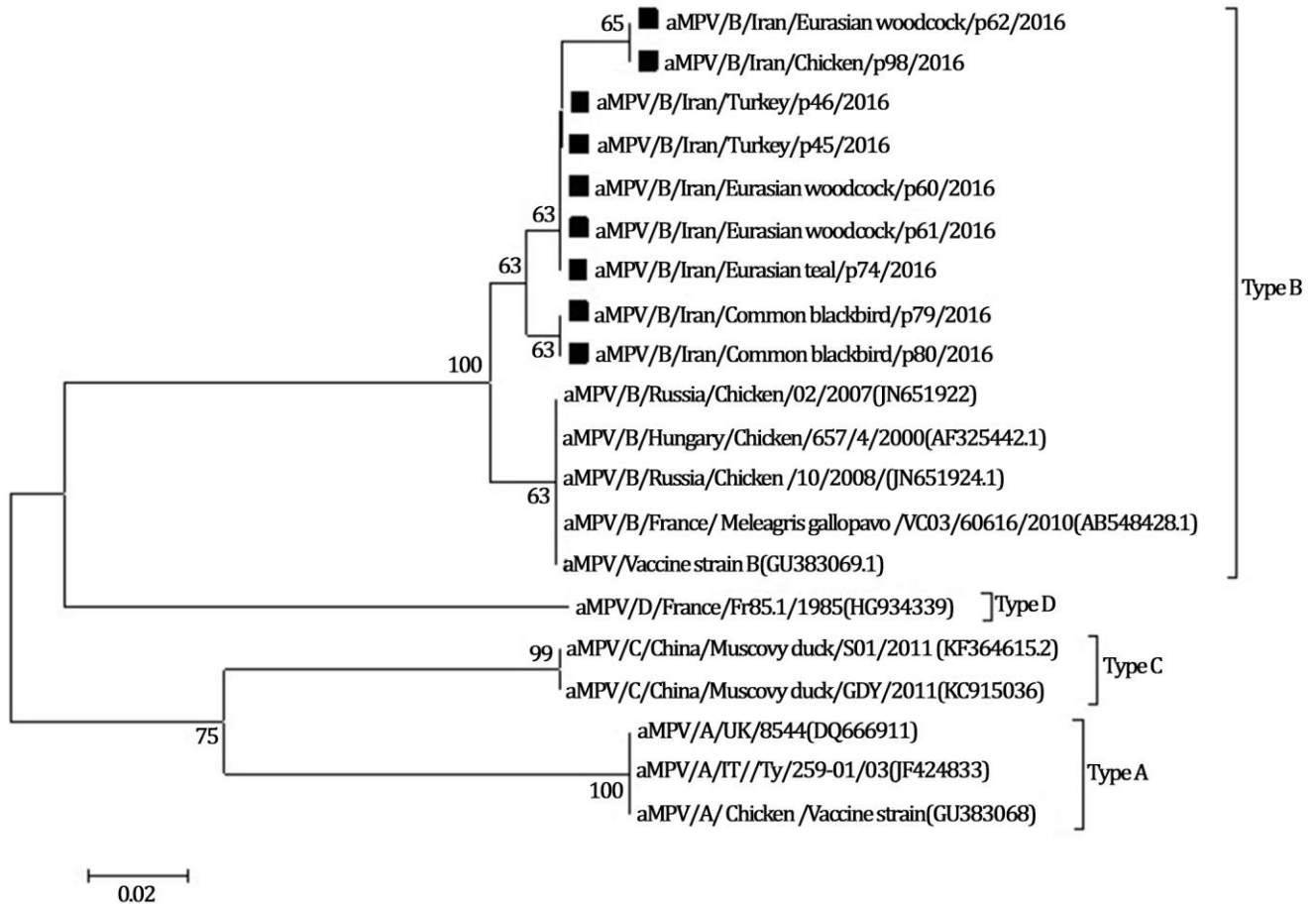
Fig. 1. Phylogenetic tree constructed based on partial N gene sequences of aMPV reference strains and field isolates. Numbers at nodes correspond to the bootstrap values (1000 replications). The aMPVs detected in the present study are marked with a black square.

Table 1. Estimated sequence identity between partial nucleotide sequences of the N gene of aMPVs detected in the examined birds and those of reference strains.

		1	2	3	4	5	6	7	8	9	10	11	12
1	aMPV/B/Iran/AnasCrecca/p74/2016	■											
2	aMPV/B/Iran/Chicken/p98/2016	97.30	■										
3	aMPV/B/Iran/Scolopaxrusticola/p61/2016	98.70	98.65	■									
4	aMPV/B/Iran/Scolopaxrusticola/p62/2016	97.30	100	98.65	■								
5	aMPV/B/Iran/Turdusmerulap/p80/2016	98.70	98.65	100	98.60	■							
6	aMPV/B/Iran/Turkey/p46/2016	98.70	98.65	100	98.60	100	■						
7	aMPV/B/France/Meleagrisgallopavo/VCO3/60616_2010(AB548428.1)	97.30	95.89	97.28	95.90	97.28	97.30	■					
8	aMPV/B/Russia/chicken/02/2007(JN651922)	97.30	95.89	97.28	95.90	97.28	97.30	100	■				
9	aMPV/B/Russia/chicken/10/2008_(JN651924.1)	97.30	95.89	97.28	95.90	97.28	97.30	100	100	■			
10	aMPV/A/IT/Ty/259-01/03(JF424833)	76.50	74.72	76.64	74.70	76.64	76.60	76.64	76.64	76.60	■		
11	aMPV/C/China/Muscovyduck/GDY/2011(KC915036)	78.50	76.71	78.53	76.70	78.53	78.50	78.53	78.53	78.50	85.30	■	
12	aMPV/vaccine strain B (GU383069.1)	80.10	78.41	80.23	78.40	80.23	80.20	80.23	80.23	80.20	80.30	74.40	■

important, especially if this involves the participation of non-domestic bird species, which would add complexity to their control on farms and implementation of vaccination and biosecurity programs for aMPV.

The first detection of aMPV in Iranian broiler flocks was published in 2010, and further studies confirmed the presence of subtype B of aMPV in Iranian commercial flocks.[3] In a study by Rahimi, anti-aMPV antibodies were detected in all examined broiler breeder flocks and 83.30% of broiler flocks.[9] Nowadays, both killed and lived attenuated pneumovirus vaccines are used in broiler, breeder and layer flocks.[3,10] Based on our unpublished data, about 40.00% to 50.00% of Iranian broiler flocks with respiratory involvements were positive for aMPV subtype B by molecular detection. In the present study, the presence of aMPV in the non-commercial birds was confirmed for the first time in Iran.

Shin *et al.* detected aMPV RNA in the samples examined from geese, sparrows, and starlings.[11] Bennett *et al.* showed that house sparrows and ring-billed gulls sampled in Minnesota and snow geese from Saskatchewan, Canada to harbor aMPV RNA.[12] Sequence analysis of aMPVs isolated from wild birds showed high amino acid sequence identity (> 97.00%) among them, and the sequence similarity between aMPvs isolated from wild bird and turkey was 93.20% to 99.30%. aMPVs were detected in wild Canada geese (66.70%) and Blue-Winged Teal (25.00%) in Canada.[13] The isolation of aMPV from wild Canada geese revealed that this species is a natural reservoir for aMPV.[13] In a study by Turpin *et al.*, antibodies against aMPVs were identified in five examined bird species including American coots, American crows, Canada geese, cattle egrets, and rock pigeons in the USA.[14] Cha *et al.* demonstrated that aMPV/C viruses carried by wild birds in the U.S. possess a potential threat to commercial turkeys.[15] In Brazil, 30.00% of all examined wild birds were positive for aMPV.[16]

There is no report regarding the presence of aMPV in the migrant and local birds that have been presented in the live bird markets of the north of Iran. There are several live bird markets in the north of Iran which present local and migrant birds coming from the north of Caspian Sea to the south. Also, this area of Iran is one of the important regions for industrial poultry farming. Several breeders, layer and broiler flocks located in this area. There is always a correlation between the health of local and migrant birds.

Studies have demonstrated the survival and loss of attenuation of vaccine strains in the environment after vaccination, which could lead to the contamination of wild birds.[17] The infection of birds other than the target species could pose different selective challenges to the vaccine virus, which, in turn, could re-infect commercial poultry with a virus with a new pathogenic profile. Interestingly, a 20-year retrospective study conducted with subtype B in Italy and other European countries revealed changes to the G protein in field strains, following the introduction of the vaccine for this subtype.[18]

This study provides further and more definite reason for maintaining high biosecurity standard in the poultry industry. Hence, we can speculate about the importance of waterfowl for the survival and evolution of aMPV in the environment. This suggests a potentially important means of dissemination of aMPV to other Iranian provinces and other countries. Also, research on the pathogenicity of the virus in wild birds is recommended.

Acknowledgments

Conflict of Interest

No conflict of interest was declared by the authors.

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