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Original Article

Molecular characterization of circulating avian metapneumovirus, subgroup B, in broiler chickens, Iran, 2016-2018

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Abstract

Background: Avian metapneumovirus (aMPV) infection has significant economic impacts on the poultry industry all around the world. **Aims:** The aim of this study is molecular investigations of different types of aMPV in broiler farms in different provinces of Iran from 2016 to 2018. **Methods:** Tracheal and oropharyngeal swabs were collected from two hundred broiler chickens with respiratory signs in ten provinces of Iran, including Kurdistan, West Azerbaijan, Semnan, Esfahan, Sistan and Baluchistan, Qazvin, Khuzestan, Fars, Gilan, and Khorasan Razavi from February 2016 to December 2018. After RNA extraction, the presence of aMPV was confirmed using *N* gene special primers. Then, subtype-specific primers were utilized to differentiate the specific subtype. All positive samples were sequenced. **Results:** As a general trend, the percentage of aMPV positive chickens increased gradually over time. All samples were clustered together and placed in the subtype B aMPV group. Although 2 samples from 2016 and 2 samples from 2018 were placed in a separate branch, most of the current study samples of 2016, 2017, and 2018 revealed six segregated sub-branches, and they were placed close to other isolates of 2011 and 2013 from Iran. **Conclusion:** The current field study indicated the presence of aMPV in a considerable number of areas in Iran. Thus, the role of this virus in broiler respiratory complex should not be neglected.

Key words: Avian metapneumovirus, Broiler, Iran, Phylogenetic analysis, RT-PCR

Introduction

Avian metapneumovirus (aMPV) can act as an important agent in multi-factorial diseases of the upper respiratory tract of turkeys and chickens. The virus can cause a swollen head syndrome in the latter host (Cecchinato *et al*., 2010). Avian metapneumovirus infection has significant economic impacts on the poultry industry all around the world (Jones, 1996). As a natural host, turkeys and chickens of any age are susceptible to aMPV infection (Umar *et al*., 2016). Usually, clinical manifestations in chickens infected solely with aMPV are milder than those in turkeys. Nonetheless, secondary bacterial and viral infection, including infectious bursal disease, Newcastle disease (ND), and infectious bronchitis, exacerbates aMPV infection signs and accelerates the development of swollen head syndrome (SHS) typical symptoms. Clinical signs in chickens are sneezing, swelling of infraorbital sinuses, ocular and nasal discharge, and opisthotonus and torticollis (Kwon *et al*., 2010; Hartmann *et al*., 2015).

Within the order of Mononegavirales, aMPV is classified as a member of the genus metapneumovirus from the Pneumoviridae family (Tucciarone *et al*., 2017b). Avian metapneumovirus has a single-stranded RNA genome with gene order 3´-N-P-M-F-M2-SH-G-L-5´ (Govindarajan *et al*., 2006; Wei *et al*., 2013). The G protein is one of the major surface glycoproteins of aMPV, which is responsible for the attachment of the virus to its receptor in cell surfaces, and acts as the major antigen of aMPV by inducing neutralizing antibodies. This protein encountered sequence divergence and is known as the most variable protein (Bäyon-Auboyer *et al*., 2000; Alvarez *et al*., 2003). However, different studies have indicated a high similarity between *G* gene sequences of isolates from the UK, Hungary, Spain, and

Italy (Cecchinato *et al*., 2010). So far, aMPV has only one recognized serotype; however, it is categorized into A, B, C, and D subtypes based on a genetic and antigenic variation of G protein (Umar *et al*., 2016). Several outbreaks of A or B subtypes in chickens and turkeys have been reported all around the world. Subtype C is isolated from game and wild birds in Europe, Asia, and America(Wei *et al*., 2013; Umar *et al*., 2016). The B subtype of aMPV was molecularly characterized in broiler and broiler breeder flocks of Iran in 2012 and 2013 (Hosseini and Ghalyanchi-Langeroudi, 2012; Ghalyanchi-Langeroudi *et al*., 2013).

Broiler farms are located in nearly all parts of Iran. However, the density of these farms is higher in some particular provinces, especially those close to the Caspian Sea in the northwest and northeast, and those in central parts of Iran. Although killed and live attenuated vaccines are given in breeder farms, serological studies showed endemic avian pneumovirus infections in Iranian poultry (Hosseini and Ghalyanchi-Langeroudi, 2012). Vaccination against aMPV is not a common procedure in broiler flocks in the country. The current study was conducted for molecular investigations of different types of aMPV in broiler farms in different provinces of Iran from 2016 to 2018.

Materials and Methods

Twenty tracheal and oropharyngeal swabs were collected from two hundred broiler flocks with respiratory signs, including swelling of infraorbital sinuses, nasal discharge, and subcutaneous edema heads in ten provinces of Iran from February 2016 to December 2018 (Table 1). The procedure has been done according to the instructor's guide and ethical standards of the University of Tehran's animals.

These flocks followed the all-in-all-out principle, and all of them had an intense rearing system.

All of the collected samples were transferred to the Laboratory of the Department of Microbiology and Immunology of the University of Tehran and were stored at -70°C until further examination. None of the sampled chickens received an aMPV vaccine.

Tracheal and oropharyngeal swabs from each farm were pooled. RNA extraction was conducted using the RNeasy Mini kit (QIAGEN, USA), and cDNA synthesis was performed by the RevertAid First-Strand cDNA Synthesis kit (Fermentas Co., Canada) according to the manufacturer's procedure (Ghafouri *et al*., 2019). The *N* gene-specific primers (Nd: 5´AGC AGG ATG GAG AGC CTC TTT G3´ and Nx: 5´CAT GGC CCA ACA TTA TGT T3´) were used to detect aMPV positive samples. To detect subtypes A and B, Ga: 5´CCG GGA CAA GTA TCT CTA TGG 3´ and G2: 5´CCA CAC TTG AAA GAT CTA CCC 3´ (*G* gene-subtype A) as well as Ga: 5´CCG GGA CAA GTA TCT CTA TGG 3´ and G12: 5´CAG TCG CCT GTA ATC TTC TAG GG3´ (*G* gene-subtype B) primers were utilized. Bäyon-Auboyer *et al*. (2000) described the polymerase chain reaction (PCR) protocols. The ABI 3100 Genetic Analyzer (Applied Biosystems, USA) and the primers (both directions) were used for sequencing (Bioneer Co., Korea).

National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) was performed to check the obtained sequences, and Bioedit software (version 7.7.9) was used to edit them (Hall, 1999; Malekan *et al*., 2016; Vasfi-Marandi *et al*., 2018). Partial *G* gene sequences of concerning subtypes from Iran and other parts of the world including Asia, Europe, and America were downloaded from the GenBank to conduct a phylogenetic analysis using the molecular evolutionary genetics analysis (MEGA) (phylogeny inference package) software (version 7.0.26). The p-distance model was used to construct a distance-based Neighbor-Joining (NJ) tree in the MEGA software (version 7.0.26) (Saitou and Nei, 1987; Nei and Kumar, 2000). 1,000 bootstrap replicates assessed phylogenetic tree robustness, and bootstrap values under 50 were omitted. The Chi-square test was used to compare the frequency of aMPV positive chickens in different years. Data were analyzed with International Business Machines Corporation (IBM) SPSS statistics software (version 24), and $P \leq 0.05$ was considered significant. The genes of the ten selected isolates submitted to GenBank and the accession numbers are MN108496, MN108497, and MN101176-MN101183.

Results

The study was conducted in ten provinces of Iran, including Kurdistan, West Azerbaijan, Semnan, Esfahan,

Table 1: Details in number of flocks sampled, number of positive samples, and percentage of positive samples in each year as well as each province

Province	2016		2017		2018		Total	
	Samples	Positive numbers (%)	Samples	Positive numbers (%)	Samples	Positive numbers (%)	Samples	Positive numbers (%)
Kurdistan	9	1 (11.1)	8	2 (25)	15	3 (20)	32	6 (18.8)
West Azerbaijan	5	2 (40)	4	1 (25)	5	4 (80)	14	7 (50)
Semnan	3	1 (33.3)	7	5 (1.4)	11	4 (36.3)	21	10 (47.6)
Esfahan	7	2 (28.5)	6	4 (66.6)	5	4 (80)	18	10 (55.6)
Sistan and Baluchistan	2	0 (0)	5	3 (60)	3	1 (33.3)	10	4 (40)
Qazvin	6	3 (50)	6	2 (33.3)	5	2 (40)	17	7 (41.2)
Khuzestan	3	2 (66.6)	2	0 (0)	4	3 (75)	9	5 (55.6)
Fars	7	1 (14.2)	6	1 (16.6)	10	6 (60)	23	8 (34.8)
Gilan	9	2 (22.2)	13	2 (15.3)	14	4 (28.5)	36	8 (22.2)
Khorasan Razavi	6	1 (16.6)	7	2 (28.5)	7	2 (28.5)	20	5 (25)
Total	57	15 (26.3)	64	22 (34.3)	79	33 (41.7)	200	70 (35)

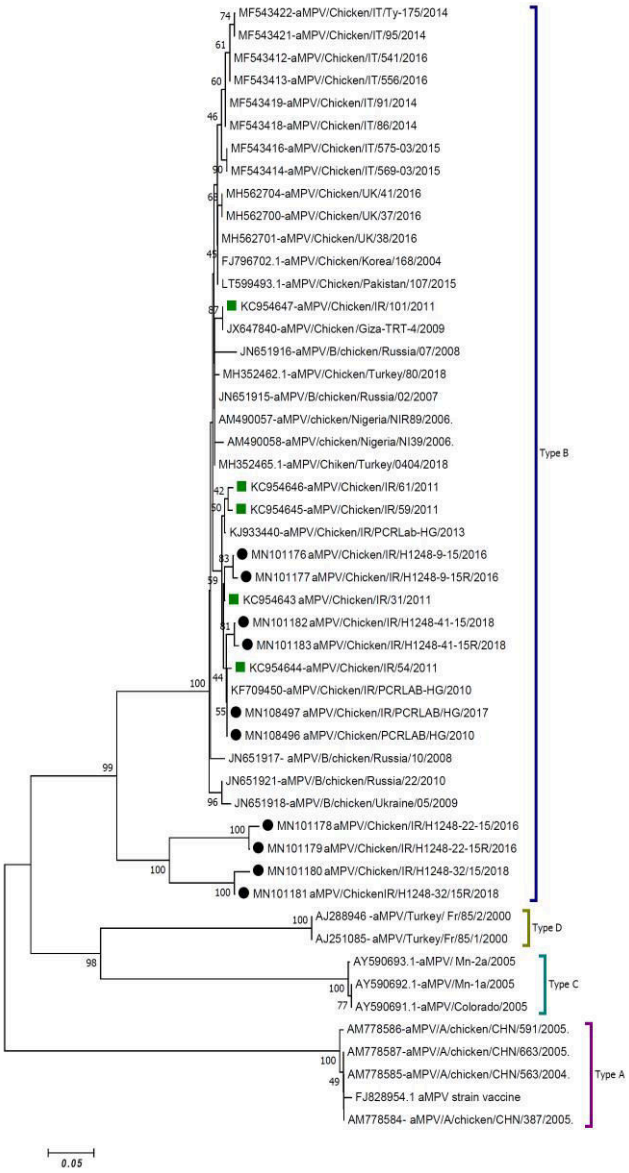

Fig. 1: The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. Bootstrap values under 50 were omitted. Evolutionary analyses were conducted in MEGA7 (black circle: current study isolates, and green square: isolates from Iran)

Sistan and Baluchistan, Qazvin, Khuzestan, Fars, Gilan, and Khorasan Razavi. Variation in the percentage of aMPV positive flocks in different years was not statistically significant (P≥0.05). Nevertheless, as a general trend, the percentage of aMPV positive flocks has increased gradually over time (Table 1). During 2016, the most positive flocks belonged to Khuzestan, Qazvin, and West Azerbaijan provinces with 66.6, 50, and 40%. However, Semnan, Esfahan, and Sistan and Baluchistan provinces exhibited the highest percentage of aMPV positive flocks in 2017. West Azerbaijan and Esfahan provinces, with 80% and Khuzestan province with 75%, possessed higher aMPV positive flocks in the last year.

All the aMPV positive samples were sequenced. As all the sequences belonging to each year possessed high similarity, ten sequences were selected to be shown here. The *G* gene phylogenic tree revealed four different groups (Fig. 1). All the Iranian isolates were clustered together and placed in the subtype B aMPV group. Within the type B group, aMPV/Chicken/IR/H1248-22-15/2016, aMPV/Chicken/IR/H1248-22-15R/2016, aMPV/Chicken/IR/H1248-32/15/2018, and aMPV/Chicken/IR/H1248-32/15R/2018 were placed in a separate branch from other Iranian samples. Furthermore, all other Iranian samples revealed six segregated sub-branches. However, aMPV/Chicken/IR/101/2011 comprised a separate branch beside Egypt's isolate. Turkey and Pakistan samples are located in different sub-branches far from Iran.

Discussion

All sequenced samples were placed in the subtype B aMPV group. Hosseini and Ghalyanchi Langeroudi (2012) detected aMPV type B from a broiler farm for the first time. In 2013, an aMPV subtype B was detected from a broiler breeder (Ghalyanchi-Langeroudi *et al*., 2013). Interestingly, a 2012 sequence of aMPV from a broiler flock and a 2013 sequence from a broiler breeder flock revealed more than 99% identity. Although the vertical transmission of aMPVs has not been confirmed so far, the identity may reflect the broiler breeder farms' role in transferring the infection to their progeny. Furthermore, breeder flocks received several live and killed aMPV vaccines, and it is assumed that this vaccination regimen will provide complete protection. Nevertheless, to overcome the bird's immune system, the aMPV field strains may evolve and increase their virulence (Jones and Rautenschlein, 2013). Moreover, based on phylogenetic analyses, it was suggested that Iran's aMPV field sample in 2010 was different from the available vaccine strain (Hosseini and Ghalyanchi-Langeroudi, 2012). Factors such as inadequate vaccination of all birds, inappropriate time, immunosuppressive and stressing agents, and incomplete vaccination programs could affect the field reversion to virulence in the live vaccine (Govindarajan *et al*., 2006; Umar *et al*., 2016; Tucciarone *et al*., 2017b). Inconsistent with the findings achieved by Hosseini and Ghalyanchi Langeroudi (2012), in Italy, none of the detected aMPV strains were phylogenetically related to vaccine strains (Tucciarone *et al*., 2017b). Furthermore, in the phylogenetic tree, aMPV/Chicken/IR/H1248-22-15/2016, aMPV/Chicken/IR/H1248-22-15R/2016, aMPV/Chicken/IR/H1248-32/15/2018, and aMPV/Chicken/IR/H1248-32/15R/2018 were placed in a separate branch from other Iranian samples. All other samples from Iran comprised of a separate cluster of six near sub-clusters, which resembles virus evolution. Maximum homology

was observed between recent sequences and other sequences reported previously in Iran (data not shown), and could be due to a common ancestor for all Iranian aMPVs. Cecchinato *et al*. (2010) reported that all recent Italian aMPV *G* genes were located in a separate cluster together, which were far from the first isolate of 1994. A phylogenetic analysis of the *G* gene from the identified aMPV strains of Italy from 2014 to 2016 also revealed an evolution in a continuous and progressive mode (Tucciarone *et al*., 2017b).

Homayounfar *et al*. (2015) investigated the presence of aMPV in the broiler, layer, and broiler breeder flocks in Iran from 2011 to 2012 by reverse transcription-polymerase chain reaction (RT-PCR). They only found 18.6% of the broiler flocks to be positive for the presence of aMPV (Homayounfar *et al*., 2015). Another study indicated that 48.1% of the broiler flocks sampled at slaughter age in the west of Iran were seropositive for aMPV (Rahimi, 2011). Additionally, 22.2% of all the sampled flocks were positive in Gilan province from 2016 to 2018. In this regard, the density of poultry farms, especially broiler breeder and broiler farms, as well as the population of rural birds, including chickens, geese, and ducks, are high in this area. Furthermore, the international wetland made this area a suitable lounge for wild migratory birds. In one study conducted in this province's live bird market, 30.6% of the sampled birds were aMPV positive (Chaboki *et al*., 2018). Moreover, it is reported that geese, starlings, sparrows, ducks, and swallows may play a significant role in spreading aMPV to neighboring farms (Shin *et al*., 2002). Thus, the role of wild and domestic reservoirs in spreading the pathogen should not be neglected. Khuzestan province had 66.6 and 75% aMPV positive flocks in 2016 and 2018, respectively. Hesami and Mayahi (2013) reported that 28% of sampled broiler flocks in Ahwaz city (capital of Khuzestan province) at slaughter age were positive for aMPV with RT-PCR. 28.8, 66.6, and 80% of sampled flocks in this province were positive for aMPV in 2016, 2017, and 2018, respectively. The density of broiler farms in one area is directly related to the rate of spread of aMPV as direct contact plays a critical role in virus transmission (Umar *et al*., 2016; Tucciarone *et al*., 2017a). In Italy, the aMPV was endemic in Italian broilers located in dense poultry farm areas (Tucciarone *et al*., 2017b).

Although most Iranian samples were clustered close together, the nearest sample from the neighboring country belongs to Turkey. Bayraktar *et al*. (2018) reported aMPV subtype B in 7.2% of broiler farms in Turkey. Due to the timing of detection, it is impossible to recognize the probability of Iranian aMPV spread to Turkey or vice versa. aMPV/Chicken/IR/101/2011 comprised a separate branch beside aMPV/Chicken/Giza-TRT-4/2009 from Egypt. We assume that migratory birds in the Black sea/Mediterranean flyway could have had a role in the transmission of Egyptian aMPV to Iran.

Based on the findings of the current comprehensive study in broiler farms and previous reports, it can be concluded that aMPV subtype B is dominant in Iran's poultry industry as there was no evidence for the presence of subtypes A, C, and D. The aMPV subtype B is present in different regions in the country, and it seems that the virus is endemic in Iran. Furthermore, the increasing number of aMPV positive flocks within three years (2016-2018) reveals the elevation field circulation of aMPVs. Thus, the role of this virus in poultry respiratory syndrome should not be underestimated. In addition to intense biosecurity implementation, aMPV vaccination in broiler farms may help as a control procedure. One dose of aMPV live-attenuated vaccine could provide lifelong protection for broilers (Cook, 2000). Nevertheless, the effectiveness of vaccination in broilers depends on the homology of the vaccine virus with the field virus, the method, age of vaccine application, and vaccination program comprehension in the region. Further investigations into layer, breeders, turkeys, and backyard poultry are needed to achieve a full epidemiological overview of Iran's aMPV infection.

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Conflict of interest

No conflict of interest is declared.

References

Alvarez, R; Lwamba, HM; Kapczynski, DR; Njenga, MK and Seal, BS (2003). Nucleotide and predicted amino acid sequence-based analysis of the avian metapneumovirus type C cell attachment glycoprotein gene: phylogenetic analysis and molecular epidemiology of US pneumoviruses. J. Clin. Microbiol., 41: 1730-1735.

Bäyon-Auboyer, MH; Arnauld, C; Toquin, D and Eterradossi, N (2000). Nucleotide sequences of the F, L and G protein genes of two non-A/non-B avian pneumoviruses (APV) reveal a novel APV subgroup. J. Gen. Virol., 81: 2723-2733.

Bayraktar, E; Umar, S; Yilmaz, A; Turan, N; Franzo, G; Tucciarone, CM; Cecchinato, M; Cakan, B; Iqbal, M and Yilmaz, M (2018). First molecular characterization of avian metapneumovirus (aMPV) in Turkish broiler flocks. Avian Dis., 62: 425-430.

Cecchinato, M; Catelli, E; Lupini, C; Ricchizzi, E; Clubbe, J; Battilani, M and Naylor, CJ (2010). Avian metapneumovirus (AMPV) attachment protein involvement in probable virus evolution concurrent with mass live vaccine introduction. Vet. Microbiol., 146: 24-34.

Chaboki, PM; Ghalyanchilangeroudi, A; Karimi, V; Abdollahi, H; Maghsoudloo, H; Hosseini, H; Farahani, RK; Ghafouri, SA; Falah, MH; Rezaee, H; Jabbarifakhr, M and Mousavi, FS (2018). Prevalence of avian metapneumovirus subtype B in live bird market in Gilan province, Iran. Vet. Res. Forum., 9: 93-97.

Cook, J (2000). Avian pneumovirus infections of turkeys and chickens. Vet. J., 160: 118-125.

Ghafouri, SA; Fallah, MH; Talakesh, SF; Hosseini, H; Ziafati, Z and Malekan, M (2019). Full genome

characterization of Iranian H5N8 highly pathogenic avian influenza virus from Hooded Crow (*Corvus cornix*), 2017: The first report. Comp. Immunol. Microbiol. Infect. Dis., 64: 73-80.

Ghalyanchi-Langeroudi, A; Haghbin-Nazarpak, H and Hosseini, H (2013). Phylogenetic study based on the gene of attachment protein (G) avian metapneumovirus from broiler breeder farm in Iran, 2013. Iran. J. Virol., 7: 7-11.

Govindarajan, D; Buchholz, UJ and Samal, SK (2006). Recovery of avian metapneumovirus subgroup C from cDNA: cross-recognition of avian and human metapneumovirus support proteins. J. Virol., 80: 5790-5797.

Hall, TA (1999). *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT*. Nucleic Acids Symposium Series. London, UK, Information Retrieval Ltd., PP: 95-98.

Hartmann, S; Sid, H and Rautenschlein, S (2015). Avian metapneumovirus infection of chicken and turkey tracheal organ cultures: comparison of virus-host interactions. Avian Pathol., 44: 480-489.

Hesami, G and Mayahi, M (2013). Detection of avian metapneumovirus infection in broilers by nested RT-PCR. Vet. Res., 17: 159-166.

Homayounfar, N; Shoushtari, H; Charkhkar, S and Bozorgmehrifard, M (2015). Detection by reverse transcriptase-polymerase chain reaction and molecular characterization of avian metapneumovirus in chicken flocks in Iran. Walia. J., 31: 170-174.

Hosseini, H and Ghalyanchi-Langeroudi, A (2012). Detection and molecular characterization of avian metapneumovirus in Iran: The first report. Iran. J. Virol., 6: 26-31.

Jones, R (1996). Avian pneumovirus infection: questions still unanswered. Avian. Pathol., 25: 639-648.

Jones, R and Rautenschlein, S (2013). Avian meta-pneumovirus. In: Swayne, DE (Ed.), *Diseases of poultry*. (13th Edn.), Iowa, John Wiley & Sons Inc., PP: 112-120.

Kwon, JS; Lee, HJ; Jeong, SH; Park, JY; Hong, YH; Lee, YJ; Youn, HS; Lee, DW; Do, SH; Park, SY; Choi, IS; Lee, JB and Song, CS (2010). Isolation and characterization of avian metapneumovirus from chickens in Korea. J. Vet. Sci., 11: 59-66.

Malekan, M; Vasfi Marandi, M; Ranjbar, MM and Bashashati, M (2016). Molecular evaluation of M2 protein of Iranian avian influenza viruses of H9N2 subtype in order to find mutations of adamantane drug resistance. Iran. J. Vet. Med., 10: 253-262.

Nei, M and Kumar, S (2000). *Molecular evolution and phylogenetics*. 1st Edn., Oxford, UK, Oxford University Press. PP: 87-111.

Rahimi, M (2011). Seroprevalence of avian metapneumovirus infection in broiler and broiler breeder chickens in Iran. Vet. Med., 56: 395-399.

Saitou, N and Nei, M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Shin, HJ; Cameron, KT; Jacobs, JA; Turpin, EA; Halvorson, DA; Goyal, SM; Nagaraja, KV; Kumar, MC; Lauer, DC; Seal, BS and Njenga, MK (2002). Molecular epidemiology of subgroup C avian pneumoviruses isolated in the United States and comparison with subgroup A and B viruses. J. Clin. Microbiol., 40: 1687-1693.

Tucciarone, CM; Andreopoulou, M; Franzo, G; Prentza, Z; Chaligiannis, I and Cecchinato, M (2017a). First identification and molecular characterization of avian metapneumovirus subtype B from chickens in Greece. Avian Dis., 61: 409-413.

Tucciarone, CM; Franzo, G; Lupini, C; Alejo, CT; Listorti, V; Mescolini, G; Brandao, PE; Martini, M; Catelli, E and Cecch (2017b). Avian Metapneumovirus circulation in Italian broiler farms. Poult. Sci., 97: 503-509.

Umar, S; Sabir, H; Ahmed, A and Subhan, S (2016). Avian metapneumovirus infection in poultry. Worlds Poult. Sci. J., 72: 833-846.

Vasfi-Marandi, M; Malekan, M; Ranjbar, MM; Davachi, ND and Alamian, S (2018). Sequencing and *in silico* multi-aspect analysis of S1 glycoprotein in 793/B serotype of infectious bronchitis virus isolated from Iran in 2003 and 2011. Arch. Razi Inst., 73: 183-198.

Wei, L; Zhu, S; Yan, X; Wang, J; Zhang, C; Liu, S; She, R; Hu, F; Quan, R and Liu, J (2013). Avian metapneumovirus subgroup C infection in chickens, China. Emerg. Infect. Dis., 19: 1092-1094.