Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.088

RESEARCH ARTICLE

Molecular Characterization of Avian Metapneumovirus from Guinea Fowls (*Numida meleagridis*)

Mattia Cecchinato[1], Caterina Lupini[2]*, Flavio Silveira[2], Valeria Listorti[2], Giulia Mescolini[2], Emilio Morandini[3], Giovanni Franzo[1] and Elena Catelli[2]

[1]Department of Animal Medicine, Production and Health (MAPS), Padua University, Agripolis - Viale dell'Università, 16 - 35020 Legnaro (PD), Italy; [2]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra, 50 40064 Ozzano dell'Emilia (BO), Italy; [3]Field Veterinary, Pescantina, VR, Italy
*Corresponding author: caterina.lupini@unibo.it

ARTICLE HISTORY (18-141)

Received: April 07, 2018
Revised: June 12, 2018
Accepted: July 24, 2018
Published online: September 27, 2018

Key words:
Avian Metapneumovirus
Guinea fowl
Molecular characterization
Phylogeny

ABSTRACT

In the present study the subtype B aMPV, strain aMPV/B/IT/GuineaFowl/1818/12, was detected in guinea fowls affected by respiratory signs, sequenced and molecularly characterized. Comparisons among several F and G gene full sequences of aMPVs subtype B, showed that no consistent pattern related to host-tropism could be identified. Moreover, analysis of partial G gene revealed the perfect identity of the guinea fowl strain with four Italian aMPVs isolated from turkeys or chickens in a time frame of three years, in the same geographic area. Phylogenetic analysis of both genes showed an evolutionary trend of subtype B circulating in Northern Italy from its first appearance in 1987, to date. The co-presence in the same geographic area of farms housing different avian species sensitive to aMPV, vaccinated with different vaccination programs or not vaccinated (like guinea fowls), and often belonging to the same integrate poultry company, could be a crucial factor for the establishment of an endemic infection.

To Cite This Article: Cecchinato M, Lupini C, Silveira F, Listorti V, Mescolini G, Morandini E, Franzo G and Catelli E, 2018. Molecular characterization of avian metapneumovirus from guinea fowls (*Numida meleagridis*). Pak Vet J, 38(4): 419-423. http://dx.doi.org/10.29261/pakvetj/2018.088

INTRODUCTION

Avian Metapneumovirus (aMPV) belongs to the family Pneumoviridae, order Mononegavirales (Rima *et al*., 2017). aMPV contain a non-segmented, negative-stranded RNA genome, of approximately 13 kb in length constituted by eight genes (3'-N-P-M-F-M2-SH-G-L-5') which encode for nine proteins. Four subtypes (A, B, C and D) have been recognized based on nucleotide sequence differences. Turkeys and chickens are the most affected species worldwide; evidence of aMPV infection has been reported in other domestic and wild birds (Cecchinato *et al*., 2016). Turkeys are the most susceptible species and the related disease is referred as Turkey rhinotracheitis (TRT). Natural and experimental infection has been reported in chickens due to subtypes A, B or C (Catelli *et al.*, 1998, 2004; Aung *et al.*, 2008; Cecchinato *et al.*, 2012; Wei *et al.*, 2013; Tucciarone *et al.*, 2018).

aMPV subtype A or B infection naturally occurs in pheasants (Catelli *et al.*, 2001) and recently subtype C has been detected too (Lee *et al.*, 2007). Waterfowls show a different subtype spectrum sensitivity. Muscovy ducks, Pekin ducks and geese seem to be sensitive only to subtype C as reported in Europe, USA and China (Cecchinato *et al.*, 2016).

Guinea fowls were shown to be sensitive to experimental infection with an un-typed strain of aMPV isolated from turkeys affected by TRT (Picault *et al.*, 1987) and to aMPV subtype A (Gough *et al*., 2002) and the virus has been isolated from field outbreaks of respiratory disease and swollen head syndrome in this species in France (Kles *et al*., 1987) and in the Netherlands (Litjens *et al*., 1989).

Moreover, clinical infection has been reported in feral guinea fowls, which were commonly found around poultry houses in South Africa, suggesting they may have played a role in the transmission of aMPV. So far molecular data on aMPV strains circulating in guinea fowls are lacking. This paper reports, for the first time, the molecular characterization of an aMPV strain detected from guinea fowls during an outbreak of respiratory disease. In order to identify if genetic differences related to the host tropism do exist, F and G genes were sequenced, aligned and compared with other aMPVs isolates from turkeys and chickens.

MATERIALS AND METHODS

Farm: Rhino-pharyngeal swabs were collected during an outbreak of respiratory disease occurred in a farm of approximately 20,000 guinea fowls, situated in a densely populated poultry area of Northern Italy (Veneto Region) and belonging to an integrated poultry company. The farm was constituted of two flocks.

Sneezing, nasal exudates, conjunctivitis and ocular discharge were observed from 70 days of age. Some birds showed oedematous periocular swelling (Fig. 1). Feed intake was reduced. Birds were treated with Colistin for 4 days. Mortality did not exceed 1%. Birds were only vaccinated for Newcastle disease (B1 strain), by spray at day-old and 22 days of age.

Fig. 1: Swollening of periorbital tissues in a guinea fowl from the flock affected by aMPV infection.

Sampling: Because of the high infectivity of aMPV in poultry, the expected prevalence was set at 30%. In each flock the population of birds was ≥3500, so 10 samples/flock were collected to be 95% certain of detecting the infection (Canon and Roe, 1982). The swabs were air-dried for approximately 30 minutes and thereafter kept at room temperature prior to be processed in pools of 10, according to the sampled flock.

Subtype specific qRT- PCR for aMPV: A subtype specific qRT-PCR based on SH gene sequence, able to differentiate A and B subtypes, was used to detect and type aMPV from dry swabs. RNA was extracted from the pools using the QIAamp® Viral RNA Mini Kit (QIAGEN S.r.l., Milan, Italy) according to the manufacturer's instructions and qRT-PCR was performed using the method described by Cecchinato *et al*. (2013b).

Sequencing of aMPV F and G genes: In order to sequence the entire F and G protein genes of the detected strain (aMPV/B/IT/GuineaFowl/1818/12), the protocol described by Cecchinato *et al*. (2010) was used. Amplified DNA products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Italia S.R.L., Milan, Italy). Sequencing was performed at Macrogen (Amsterdam, the Netherland).

Phylogenetic analysis: Nucleotide sequences were edited and assembled using Bioedit software and aligned against analogous gene sequences of aMPV subtype B isolated in Italy or published on GenBank, using Clustal W. Independent phylogentic trees were reconstructed for the complete F and G gene using the Maximum likelihood (ML) approach implemented in PhyML (Guindon *et al.*, 2010). The best substitution model was selected based on the Akaike information criteria (AIC), calculated using JmodelTest (Darriba *et al.*, 2012). The best tree search method included the combination of two branch swapping algorithms: nearest neighbor interchange (NNI) and subtree pruning and regrafting (SPR). The branch support was evaluated using 1000 bootstrap replicates. Raw genetic distances among sequence pairs and the related heatmaps were calculated using the *ape* (Paradis *et al.*, 2013) and *phytools* (Revell, 2012) R libraries, respectively.

To benefit of a higher sequences number, a phylogenetic tree was also reconstructed based on a partial G gene alignment using the previously described approach and a slightly modified version of the sequence dataset (Tucciarone *et al.*, 2017).

RESULTS

The aMPV subtype B was detected from both bird flocks. The sample that showed the lower cp by qRT-PCR was named aMPV/B/IT/GuineaFowl/1818/12 and was selected for attempting the molecular characterization of the complete F and G genes. The nucleotide sequences obtained were deposited in GenBank with the following accession numbers: KC542808 (F gene) and KC960435 (G gene).

The phylogenetic trees based on complete nucleotide or amino acid sequences of F and G genes showed a similar topology, characterized by the presence of one main cluster comprising all Italian strains isolated after 2001, including the aMPV detected from guinea fowl (aMPV/B/IT/GuineaFowl/1818/12) (Fig. 2).

In the G gene, strain IT/GuineaFowl/1818/12 showed the highest homology with the Italian strain IT/Ty/2009/13 (99.8% of homology). A lower homology, ranging from 98.1 to 99.7% was observed with other Italian strains isolated after 2001 (Table 1). Italian strains detected before 2001 were genetically more distant (homology ≤97.6%). Similar results were obtained comparing F genes sequences (Table 1). The comparison of the guinea fowl strain with the other Italian aMPVs demonstrated a decrease in genetic identity, which correlates to the strain isolation year. A higher distance was observed with viruses isolated in Russia (93.4-97.3% of homology), Ukraine (94.3%) and Israel (95.6%).

Comparison of partial G gene was moreover performed, due to the availability of larger number of sequences. Results showed the perfect identity of the guinea fowl strain with four Italian aMPVs isolated from turkeys (strain IT/Ty/2009/13) or chickens (strains IT/Ck/569-03/2015, IT/Ck/575-03/2015 and IT/Ck/575-04/2015) in a time frame of three years, in the same geographic area (Fig. 3). These strains were part of a cluster including other Italian viruses (from 2001-2013) and aMPVs detected in Spain and Greece in 2016 (Fig. 3).

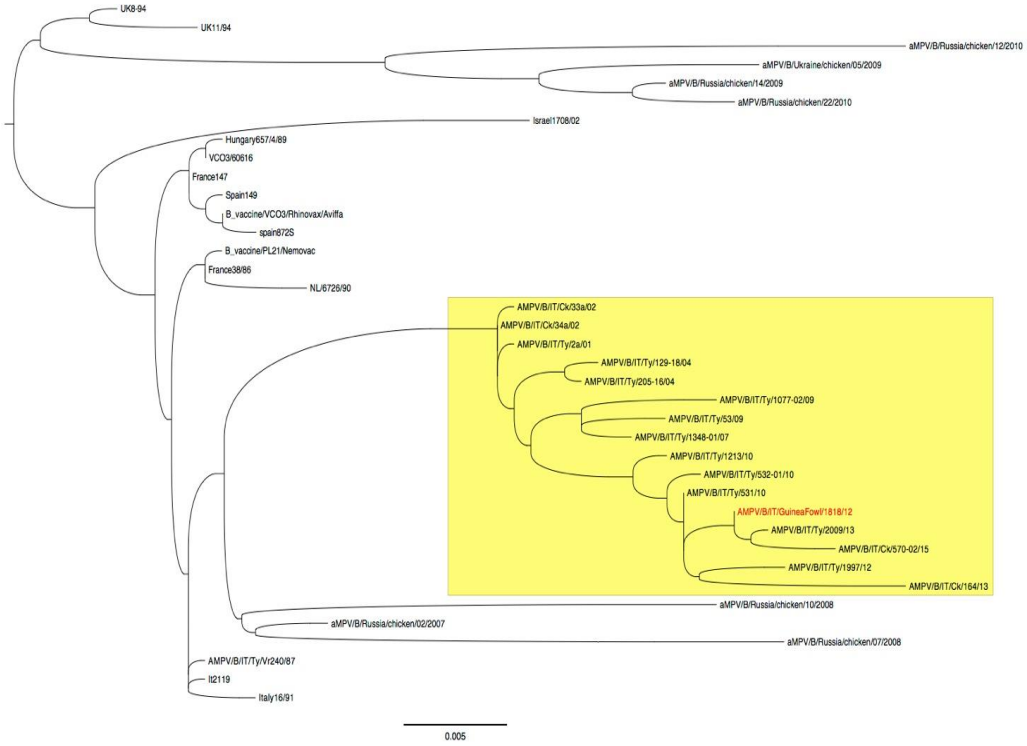

Fig. 2. Phylogenetic tree based on the alignment of complete G gene nucleotide sequences of subtype B AMPVs, constructed using the maximum likelihood method implemented in the PhyML software. A yellow box highlights a main cluster comprising all Italian strains isolated after 2001, including the aMPV detected from guinea fowl (red font).

Table 1: Percentage of nucleotide (nt) and amino acid (AA) homology between G and F gene sequences of the strain aMPV/B/IT/GuineaFowl/1818/12 and aMPV subtype B Italian strains isolated from 1987 to 2015

Strain	Year of detection	Homology (%)			
		G gene		F gene	
		nt	AA	nt	AA
aMPV/B/IT/Ty/Vr240/87	1987	97.6	95.3	97.5	98.5
It2119	NA	97.6	95.3	NA	NA
Italy16/91	1991	97.4	95.3	97.4	98.8
aMPV/B/IT/Ty/2a/01	2001	98.7	97.4	98.7	99.0
aMPV/B/IT/Ck/33a/02	2002	98.7	97.4	98.6	98.8
aMPV/B/IT/Ck/34a/02	2002	98.8	97.6	98.5	98.7
aMPV/B/IT/Ty/129-18/04	2004	98.4	97.4	98.5	98.7
aMPV/B/IT/Ty/205-16/04	2004	98.5	97.1	98.5	98.8
aMPV/B/IT/Ty/1348-01/07	2007	98.4	97.4	NA	NA
aMPV/B/IT/Ty/53/09	2009	98.5	97.9	99.2	99.4
aMPV/B/IT/Ty/1077-02/09	2009	98.1	97.6	99.2	99.4
aMPV/B/IT/Ty/1213/10	2010	99.3	98.9	NA	NA
aMPV/B/IT/Ty/531/10	2010	99.7	99.4	99.5	99.6
aMPV/B/IT/Ty/532-01/10	2010	99.4	99.2	99.5	99.6
aMPV/B/IT/Ty/1997/12	2012	99.2	98.4	99.5	99.4
aMPV/B/IT/Ty/2009/13	2009	99.8	99.7	99.6	99.4
aMPV/B/IT/Ck/164/13	2013	98.6	97.4	99.3	99.0
aMPV/B/IT/Ck/570-02/15	2015	99.4	98.9	99.5	99.4
IT/Ck/2060/13	2013	NA	NA	99.2	99.4
Italy.182.88	NA	NA	NA	97.2	98.3
IT/Ty/132-08/04	2004	NA	NA	97.7	98.5
IT/Ty/129-08/04	2004	NA	NA	97.7	98.5

NA=Not available.

DISCUSSION

In the present study a subtype B aMPV strain (aMPV/B/IT/GuineaFowl/1818/12) detected in guinea fowls affected by respiratory signs was sequenced and molecularly characterized. To our knowledge, this is the first time that sequences of an aMPV from guinea fowls are made available to the scientific community and compared with strains originating from different species.

F and G genes of the analysed strain were 1,617bp or 1,242 bp in length, respectively, as reported for aMPVs of subtype B isolated from turkey and chickens (Cecchinato *et al.*, 2010). Comparisons among several F and G gene full sequences of aMPVs subtype B, showed that while numerous individual sequence differences between viruses isolated in different avian species are present, no consistent pattern related to host-tropism could be identified. In particular, the guinea fowl aMPV strain showed high homology with aMPVs subtype B isolated from turkeys or chickens in the specific geographic area, indicating that the strains do not possess peculiar differences linked to the species of origin since a substantial identity among aMPV sequences detected in the same period from broiler, turkeys and guinea fowls. Therefore, it is likely that similar strains are circulating in more than a single target species. Moreover, phylogenetic analysis of both genes shows an evolutionary trend of subtype B circulating in Northern Italy from its first appearance in 1987 to 2015 (to which date back our latest sequences), as previously reported (Cecchinato *et al.*, 2010, Tucciarone *et al.*, 2018). It is likely that the Italian aMPVs subtype B are accumulating progressive mutations from a common progenitor, potentially because of vaccine induced immune pressure (Catelli *et al.*, 2010). Furthermore, the co-presence in the same geographic area of farms housing different avian species sensitive to aMPV, vaccinated with different vaccination programs or not vaccinated (like guinea fowls), and often belonging to the same integrate poultry company, could be a crucial factor for the establishment of an endemic infection (Cecchinato *et al.*, 2013a), reversion to virulence of vaccines and environmental spread of vaccine-derived strains (Catelli *et al.*, 2006; Brown *et al.*, 2011; Lupini *et al.*, 2011; Cecchinato *et al.*, 2014; Listorti *et al.*, 2014).

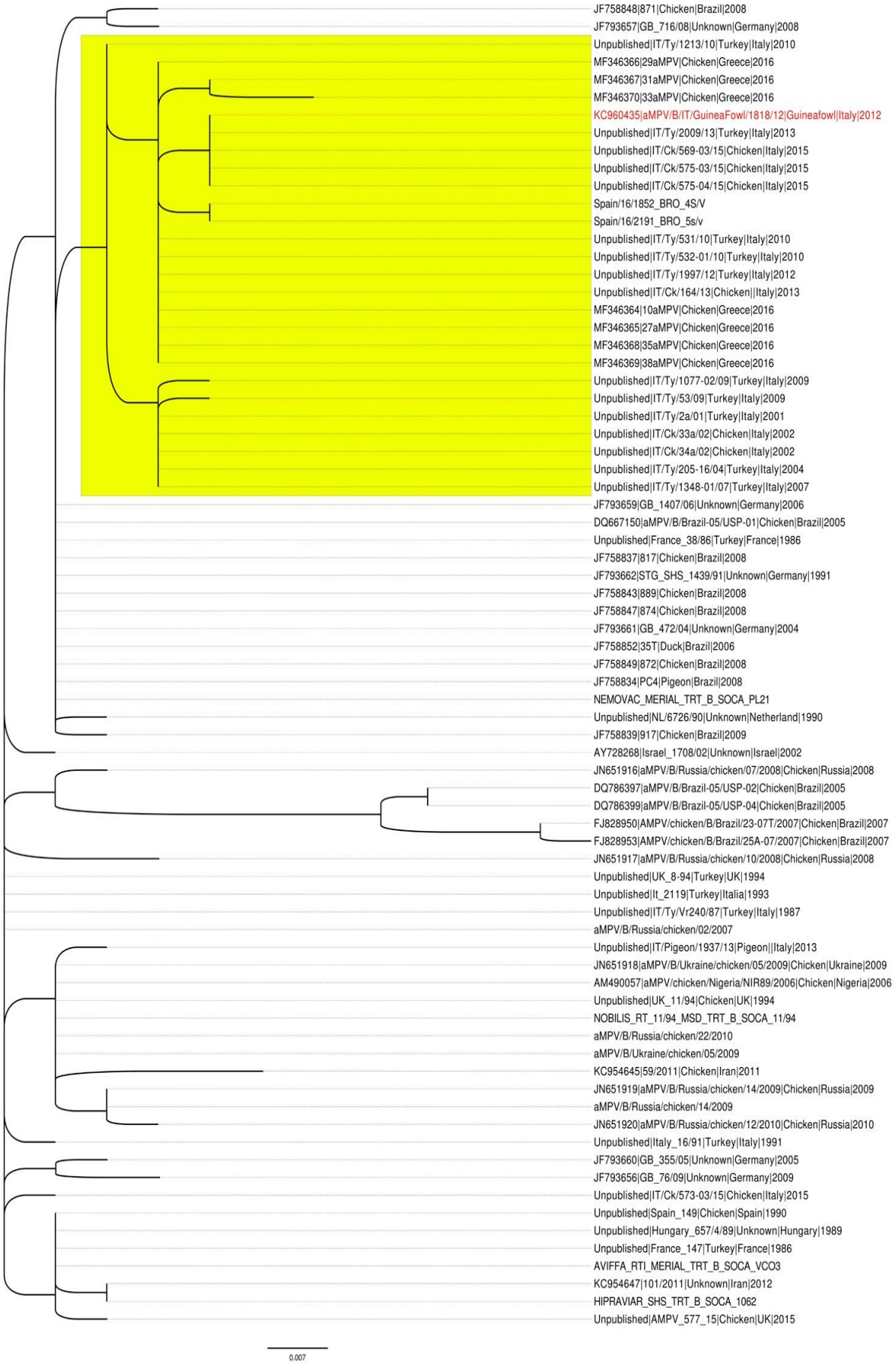

Fig. 3: Phylogenetic tree based on the alignment of partial G gene nucleotide sequences of subtype B AMPVs, constructed using the maximum likelihood method implemented in the PhyML software. A yellow box highlights a main cluster comprising Italian strains isolated after 2001, including the aMPV detected from guinea fowl (red font), Greeks and Spanish strains detected in 2016.

Authors contribution: MC, CL and EC conceived and designed the experiments; FS, VL, GM and EM performed the experiments; GF analyzed the data; CL and GF reviewed the manuscript; MC and EC wrote the paper.

REFERENCES

Aung YH, Liman M, Neumann U, *et al.*, 2008. Reproducibility of swollen sinuses in broilers by experimental infection with avian metapneumovirus subtypes A and B of turkey origin and their comparative pathogenesis. Avian Pathol J WVPA 37:65-74.

Brown PA, Lupini C, Catelli E, *et al.*, 2011. A single polymerase (L) mutation in avian metapneumovirus increased virulence and partially maintained virus viability at an elevated temperature. J Gen Virol 92:346-54.

Canon RM and Roe RT, 1982. Livestock disease surveys: a field manual for veterinarians. Australian Bureau of Animal Health. Australian Government Publishing Service, Canberra, Australia 17.

Catelli E, Cook JK, Chesher J, *et al.*, 1998. The use of virus isolation, histopathology and immunoperoxidase techniques to study the dissemination of a chicken isolate of avian pneumovirus in chickens. Avian Pathol 27:632-40.

Catelli E, Terregino C, De Marco MA, *et al.*, 2001. Serological evidence of avian pneumovirus infection in reared and free-living pheasants. Vet Rec 149:56.

Catelli E, Cecchinato M, Cassandro M, *et al.*, 2004. Avian Pneumovirus infection in turkey and broiler farms in Italy: a virological, molecular and serological field survey. Ital J Anim Sci 3:287-92.

Catelli E, Cecchinato M, Savage CE, *et al.*, 2006. Demonstration of loss of attenuation and extended field persistence of a live avian metapneumovirus vaccine. Vaccine 24:6476-82.

Catelli E, Lupini C, Cecchinato M, *et al.*, 2010. Field avian Metapneumovirus evolution avoiding vaccine induced immunity. Vaccine 28:916-21.

Cecchinato M, Catelli E, Lupini C, *et al.*, 2010. Avian metapneumovirus (AMPV) attachment protein involvement in probable virus evolution concurrent with mass live vaccine introduction. Vet Microbiol 146:24-34.

Cecchinato M, Lupini C, Ricchizzi E, *et al.*, 2012. Italian field survey reveals a high diffusion of avian metapneumovirus subtype b in layers and weaknesses in the vaccination strategy applied. Avian Dis 56:720-4.

Cecchinato M, Drigo M, Lupini C, *et al.*, 2013a. Diffusione dell'infezione da metapneumovirus aviare in allevamenti di tacchini e broiler nel Nord Italia. Large Anim Rev 19:267-70.

Cecchinato M, Lupini C, Munoz Pogoreltseva OS, *et al.*, 2013b. Development of a real-time RT-PCR assay for the simultaneous identification, quantitation and differentiation of avian meta-pneumovirus subtypes A and B. Avian Pathol 42:283-9.

Cecchinato M, Catelli E, Lupini C, *et al.*, 2014. Reversion to virulence of a subtype B avian metapneumovirus vaccine: Is it time for regulators to require availability of vaccine progenitors? Vaccine 32:4660-4.

Cecchinato M, Ferreira HL, Munir M, *et al.*, 2016. Avian metapneumovirus. Mononegaviruses of veterinary importance. In: Molecular epidemiology and control: Vol 2 (Munir M, ed): CABI Publishing, Wallingford, UK pp:127-43.

Darriba D, Taboada GL, Doallo R, *et al.*, 2012. JmodelTest 2: more models, new heuristics and high-performance computing. Nat Methods 9:772-2.

Gough RE, Drury SE, Welchman D de B, *et al.*, 2002. Isolation of birnavirus and reovirus-like agents from penguins in the United Kingdom. Vet Rec 151:422-4.

Guindon S, Dufayard JF, Lefort V, *et al.*, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307-21.

Kles V, Morin M, Picault JP, *et al.*, 1987. Isolement d'un virus dans un cas de syndrome infectieux du gonflement de la tete avec coryza chez la pintade. L'aviculteur 483:112.

Lee EH, Song MS, Shin JY, *et al.*, 2007. Genetic characterization of avian metapneumovirus subtype C isolated from pheasants in a live bird market. Virus Res 128:18-25.

Listorti V, Lupini C, Cecchinato M, *et al.*, 2014. Rapid detection of subtype B avian metapneumoviruses using RT-PCR restriction endonuclease digestion indicates field circulation of vaccine-derived viruses in older turkeys. Avian Pathol 43:51-6.

Litjens JB, van Willigen FC and Sinke M, 1989. A case of swollen head syndrome in a flock of guinea fowl. Tijdschr Diergeneeskd 114:719-20.

Lupini C, Cecchinato M, Ricchizzi E, *et al.*, 2011. A turkey rhinotracheitis outbreak caused by the environmental spread of a vaccine-derived avian metapneumovirus. Avian Pathol 40:525-30.

Paradis E, Bolker B, Claude J, *et al.*, 2013. Ape: Analyses of phylogenetics and evolution. Bioinformatics 20:289-90.

Picault JP, Giraud P, Drouin P, *et al.*, 1987. Isolation of a TRTV-like virus from chickens with swollen-head syndrome. Vet Rec 121:135.

Revell LJ, 2012. Phytools: An R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3:217-23.

Rima B, Collins P, Easton A, *et al.*, 2017. ICTV Virus Taxonomy Profile: Pneumoviridae. J Gen Virol 98:2912-3.

Tucciarone CM, Andreopoulou M, Franzo G, *et al.*, 2017. First Identification and molecular characterization of avian metapneumovirus subtype B from chickens in Greece. Avian Dis 61:409-13.

Tucciarone CM, Franzo G, Lupini C, *et al.*, 2018. Avian Metapneumovirus circulation in Italian broiler farms. Poult Sci 97:503-9.

Wei L, Zhu S, Yan X, *et al.*, 2013. Avian metapneumovirus subgroup C infection in chickens, China. Emerg Infect Dis 19:1092-4.