Pertanika J. Trop. Agri. Sci. 46 (3): 971 - 1002 (2023)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Review Article

Avian Metapneumovirus Infection in Poultry Flocks: A Review of Current Knowledge

Wafaa A. Abd El-Ghany

Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt

ABSTRACT

Avian metapneumovirus (aMPV) is one of the respiratory viruses that cause global economic losses in poultry production systems. Therefore, it was important to design a comprehensive review article that gives more information about aMPV infection regarding the distribution, susceptibility, transmission, pathogenesis, pathology, diagnosis, and prevention. The aMPV infection is characterized by respiratory and reproductive disorders in turkeys and chickens. The disease condition is turkey rhinotracheitis in turkeys and swollen head syndrome in chickens. Infection with aMPV is associated with worldwide economic losses, especially in complications with other infections or poor environmental conditions. The genus Metapneumovirus is a single-stranded enveloped RNA virus and contains A, B, C, and D subtypes. Meat and egg-type birds are susceptible to aMPV infection. The virus can transmit through aerosol, direct contact, mechanical, and vertical routes. The disease condition is characterized by respiratory manifestations, a decrease in egg production, growth retardation, increasing morbidity rate, and sometimes nervous signs and a high mortality rate, particularly in concurrent infections. Definitive diagnosis of aMPV is based mainly on isolation and identification methods, detection of the viral DNA, as well as seroconversion. Prevention of aMPV infection depends on adopting biosecurity measures and vaccination using inactivated, live attenuated, and recombinant or DNA vaccines.

Keywords: Distribution, pathology, swollen head syndrome, turkey rhinotracheitis, vaccine

ARTICLE INFO

Article history:
Received: 01 February 2023
Accepted: 21 February 2023
Published: 30 August 2023

DOI: https://doi.org/10.47836/pjtas.46.3.14

E-mail address:
wafaa.soliman@cu.edu.eg (Wafaa Abd El-Ghany)

INTRODUCTION

Respiratory viral diseases are the primary causes of severe economic losses in poultry production systems. Avian metapneumovirus (aMPV) infection is regarded as a significant and highly contagious respiratory viral

ISSN: 1511-3701
e-ISSN: 2231-8542

disease (Tucciarone et al., 2018). Infection with aMPV is associated with poor feed conversion ratio, a drop in egg production with alterations of egg quality, and mortalities, especially in complications.

In 1978 in South Africa, aMPV induced a disease condition in turkey named 'turkey rhinotracheitis' (TRT) (Buys et al., 1989). However, in 1984 in France and the United Kingdom, the same virus was first termed in chickens and described as 'swollen head syndrome' (SHS) (Morley & Thomson, 1984). In the 1980s, aMPV was detected in turkeys and commercial fowl in some European countries and elsewhere in the 1990s, such as the United States of America (USA). The previous infections aMPV induced were avian infectious pneumoniae, avian pneumovirus, and avian rhinotracheitis (Alkahalaf et al., 2002).

The virus mainly affects turkeys, chickens, and other avian species with variable mortality rates (Brown et al., 2019; Tucciarone et al., 2022). Air is the main method of aMPV transmission. However, direct contact with wild birds is possible (Alkahalaf et al., 2002). Concurrent secondary infections dramatically exacerbate the virus infection. *Escherichia coli*, *Bordetella avium*, *Ornithobacterium rhinotracheale* (ORT), *Riemerella anatipestifer*, *Mycoplasma gallisepticum*, *Avibacterium paragallinarum*, *Chlamydophila psittaci*, Newcastle disease virus (NDV), infectious bronchitis virus (IBV), avian orthoavulavirus-1, infectious laryngotracheitis virus, or *Aspergillus fumigatus* can considerably enhance the incidence and severity of aMPV infection (Croville et al., 2018). The aMPV infections are globally distributed in poultry-producing areas with adverse economic losses. Based on reactivity against monoclonal antibodies, serological tests, and nucleotide sequence analysis, subtypes A, B, C, and D of aMPV are found to be antigenically distinctive (Cook et al., 1993). Recent sequence analysis of the virus showed the presence of additional subtypes in wild and game birds of North America. Based on L gene sequences, the phylogenetic analysis of aMPV revealed that the new subtypes are close to the subgroup C (Retallack et al., 2019; World Organisation for Animal Health [WOAH], 2022). Vaccination against aMPV has been adopted to protect flocks from infection with variable immune responses. Several attempts have been carried out to develop inactivated, living attenuated, and recombinant vaccines against aMPV infection in commercial turkey and chicken flocks (Śmiałek et al., 2021).

This review article was designed to give more information about aMPV infection regarding distribution, susceptibility, transmission, pathogenesis, pathology, diagnosis, and prevention.

THE VIRUS

Avian metapneumovirus is a member of *Mononagavirales*, the family *Paramixoviridae*, the sub-family *Pneumoviridae*, and the genus *Metapneumovirus* (Pringle, 1998). There are two genera in the family of *Pneumoviridae*: the *Orthopneumovirus* and

the *Metapneumovirus*. The virus is a single-stranded, negative-sense RNA, enveloped, linear, non-segmented, and pleomorphic (80 to 200 nm) or spherical. The viral genome in a nucleocapsid is about 14 kilo-bases with a helical symmetry (M. Yu et al., 2019). The viral gene order is 3′-leader-N-P-M-F-M2-SH-G-L-trailer-5′ (Ling & Pringle, 1988). Moreover, the aMPV characteristics differ from mammalian pneumoviruses at the molecular level (Kuhn et al., 2020). As aMPV has no hemagglutination or neuraminidase activity in G attachment protein, it could be distinguished from other paramyxoviruses.

Avian metapneumovirus has 4 subtypes: A, B, C, and D, according to the nucleotide and amino acids sequences of the G attachment gene as well as the serological properties (Chacón et al., 2007), and they are circulating in many countries all over the world. Subtypes A and B of aMPV were first recognized in the 1980s (McDougall & Cook, 1986), and they are prevalent, especially in Europe (Giraud et al., 1986; Naylor, Shaw, et al., 1997). However, the field reports of aMPV pointed to the higher incidence of subtype B over subtype A in some countries for unclear reasons (Chacón et al., 2011). Moreover, sub-populations of subtype B may be found due to escaping of vaccine mutants with inadequate protection. Subtype C of the virus was demonstrated in turkey flocks of the USA (Seal, 1998) and wild avian populations (Turpin et al., 2008). Moreover, subtype C was detected in breeder flocks of ducks in France and China (Wei et al., 2013). In Europe, a second distinctive lineage of subtype C has been found (Toquin et al., 2006). In the USA, though strains of aMPV are classified as subgroup C, a novel subgroup showed a higher genetic similarity to human MPV than avian types (Govindarajan & Samal, 2004). Later, subtype C was distributed in France (Toquin et al., 2006) and South Korea in avian species. Also, in France (Bäyon-Auboyer et al., 2000) and other countries (Cook et al., 2000), subtype D of aMPV was isolated from turkeys. Subtypes A, B, and D of the virus are nearly similar.

The presence of an envelope in aMPV increases the sensitivity of the virus to the lipid solvents and some physical and chemical agents (Townsend et al., 2000). The viability of aMPV can be decreased after exposure to some disinfectants including ethanol, quaternary ammonium compounds, iodophor, phenol, and sodium hypochlorite (L. Zhang et al., 2002). The virus may remain active for up to 26 weeks at -20°C and 60 days at 12°C (Velayudhan et al., 2003).

DISTRIBUTION

Since aMPV was isolated in the late 1970s, the virus has been distributed worldwide. The virus infection is characterized by a rapid spread and distribution within the flock and between flocks such as those of European countries (Franzo et al., 2020; Mescolini et al., 2021). The distribution of aMPV depends on hygienic measures, shedding rate, seasonal variation, and stocking density (Jardine et al., 2018). Despite the development of management and hygiene measures and vaccination programs, the incidence of aMPV is still

high in many countries and continents worldwide, including Japan (Mase et al., 2003), Israel (Banet-Noach et al., 2005), Korea (Kwon et al., 2010), China (S. Sun et al., 2014), Romania (Franzo et al., 2017), Greece (Tucciarone et al., 2017), Bangladesh (Ali et al., 2019), Brazil (Rizotto et al., 2019), Pakistan (Umar et al., 2019), and Italy (Graziosi et al., 2022; Legnardi et al., 2021).

There are limited data about the incidence of aMPV in the Middle East region, possibly due to other more significantly important respiratory viruses. However, the virus was detected in some of the Middle East countries such as Egypt, Iraq, Iran, Jordan, Morocco, Algeria, and Turkey (Table 1).

Table 1

The incidence of avian metapneumovirus in some countries of the Middle East region

Country	History	Findings	Method of detection	Reference
Egypt	Serum samples were collected from 30-38-week-old broiler breeder chickens	Six of 38 serum samples were serologically positive for aMPV	ELISA	Youssef and Ahmed (1996)
	Seventy-five serum samples were taken from 20 and 52-day-old broiler chickens representing 15 farms in different provinces. Ten farms had respiratory signs, and the other 5 farms were apparent healthy	High seroprevalences to aMPV were found in the 14 flocks. Antibodies were detected in broilers from apparent healthy farms	ELISA	Aly et al. (1997)
	Ten oropharyngeal swabs were collected from 3-week-old turkey poults with upper respiratory disease in Fayoum province	Subtype A of aMPV was identified	RT-PCR	Abdel-Azeem et al. (2014)
	Oropharyngeal and nasal sinus swabs were taken from 100 broiler turkeys representing 10 farms and showed respiratory signs in Giza, Beni-Suif, and Cairo provinces	Subtype B of aMPV strains was detected in 8 samples, which were very close to the VCO3 vaccine strain	Embryonated chicken eggs, Vero cells, RT-PCR, and genetic analysis	Arafa et al. (2015)

Table 1 *(Continue)*

Country	History	Findings	Method of detection	Reference
	Serum samples were taken from 40 chicken flocks (23 broilers and 17 layers), and 8 duck flocks (6 Pekin and 2 Muscovy)	Five out of 23 broilers, 6 out of 17 layers, 1 out of 6 Pekin ducks, and 1 out of 2 Muscovy ducks were serologically positive for aMPV	ELISA	Nagy et al. (2018)
Egypt	Trachea, lung, and choanal cleft were collected from broiler chicken flocks in different provinces	Subtype B of aMPV was detected. In addition to co-infection with *E. coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*	RT-PCR and real-time polymerase chain reaction (qPCR) for viral detection. Besides conventional bacteriological examination and PCR	Abdelmoez et al. (2019)
	Swabs from the trachea, sinuses, lungs, and air sacs were collected from 67 3-6-week-old broiler chickens with SHS in Baghdad, Wasit, Karbal, Al Muthanna, Al-Najaf, and Al-Qadisiyyah provinces	Detection of ORT as one of the etiological factors that cause SHS in poultry	Conventional bacteriological examination and RT-PCR	Al-Hasan et al. (2021)
Iraq	Sixty-seven swabs from the trachea, sinuses, lungs, and air sacs were taken from 3-6-week-old broiler farms in Baghdad, Wasit, Karbal, Al Muthanna, Al-Najaf, and Al-Qadisiyyah provinces	Subtype B of aMPV was found in 16 (23.8%) samples; 51 (76.11%) were negative from typical SHS-infected flocks, and no positive samples for other subtypes were found	RT-PCR	Al-Hasan et al. (2022)

Table 1 *(Continue)*

Country	History	Findings	Method of detection	Reference
Iran	A total of 540 serum samples were collected from 27 broiler breeder flocks in 11 provinces	Serologically, 92.59% were positive, while 2 were suspected of aMPV infection. Besides, 92.77% were positive, 3.70% were suspected, and the others were negative	ELISA	Sheikhi and Masoudian (2011)
	Oropharyngeal and turbinate swabs were collected from un-vaccinated 4 broiler chickens with swollen heads, and 10% mortality in Alborz province	Subtype B of aMPV was isolated	RT-PCR	Hosseini and Ghalyanchi-Langeroudi (2012)
	A total of 525 blood samples and trachea/ nasal turbinates swabs were taken from 35 non-vaccinated broiler flocks with respiratory signs in northern Iran	Ten (28.5%) of flocks had positive antibodies to aMPV. Of the 35 flocks, 8 (23%) were positive aMPV	ELISA and RT-PCR	Seifi et al. (2015)
	Tissue samples of broiler chickens were taken	Subtype B of aMPV was identified based on the fusion (F) gene	RT-PCR	Hosseini et al. (2017)
	Sixty-three meat-type unvaccinated turkey flocks from several provinces were sampled in major turkey abattoirs	Twenty-six samples from three flocks (4.10%) were positive for the virus RNA, while all viruses were detected as aMPV subtype B	RT-PCR	Mayahi et al. (2017)

Table 1 *(Continue)*

Country	History	Findings	Method of detection	Reference
Iran	Tracheal swabs were collected from 20 broiler chicken farms that had respiratory disease complex in Qazvin province	Thirteen out of 20 flocks were infected with aMPV, which accounted for 65 infection rates of the flocks	RT-PCR	Zahirabadi et al. (2017)
	Four hundred and fifty oropharyngeal samples were taken from 8 migratory and local species of birds from live bird markets in Gilan province	The aMPV subtype B was detected in 30.60%, including chickens (37%), turkeys (33%), Eurasian teal (25%), common blackbirds (33%), and Eurasian woodcock (25%)	RT-PCR	Chaboki et al. (2018)
	Samples from the trachea, choana, and sinuses of more than 3-week-old broiler chickens were collected from 85 broiler flocks in Semnan province	Thirty out of 85 (35.3%) flocks were positive for aMPV. Besides, 28 positive samples were subtype B, and the other 2 were non-subtype B, possibly A, C, or D	RT-PCR	Darebaghi et al. (2021)
Jordan	In northern and central Jordan, 38 chicken flocks (23 broilers, 8 layers, and 7 broiler breeders) were tested for serology. However, 150 chicken flocks (133 broiler flocks, 7-layer flocks, and 10 broiler breeder flocks) were tested for molecular virus detection	Antibodies against aMPV were detected in 5 of 23 broiler chickens (21.7%), 6 of 8-layer chickens (75%), and 7 of 7 (100%) broiler breeder chickens. Molecularly, aMPV was detected in 17 broiler flocks (12.8%) and 3-layer flocks (42.9%). All the broiler breeder chickens were negative. All aMPV isolates were subtype B	ELISA and RT-PCR	Gharaibeh and Algharaibeh (2007)

Table 1 *(Continue)*

Country	History	Findings	Method of detection	Reference
Jordan	Trachea swabs from 115 diseased broiler chicken flocks were tested for avian influenza virus (AIV) subtype H9N2, IBV, NDV, and aMPV	Thirteen and 14.8% were infected with NDV and IBV, respectively, whereas 5.2, 6.0, 9.6, 10.4, 11.3, and 15.7% were infected with *Mycoplasma gallisepticum (*MG) and NDV; aMPV and MG; NDV and IBV; MG and IBV; AIV and NDV; as well as AIV and IBV, in a respective manner. Moreover, 2.6% showed aMPV, NDV, and IBV infections. However, 11.3% were negative for all these respiratory viruses	PCR and RT-PCR	Roussan et al. (2008)
Morocco	Serum samples were taken from 48, 5-week-old non-vaccinated broiler chicken flocks in different bioclimatic zones	From 1,142 sera, 912 (79.86%) samples were positive serologically to aMPV. The arid zone had the highest seroprevalences. Moreover, 94.16% during winter and 84.82% during spring were positive	ELISA	Mernizi et al. (2022)
Algeria	Tissues were collected from the respiratory tract of broiler chickens	Subtype B of aMPV was detected with IBV, AIV, and MG	RT-PCR	Sid et al. (2015)

Table 1 *(Continue)*

Country	History	Findings	Method of detection	Reference
Turkey	A total of 624 tracheal samples were taken from a local turkey abattoir. Besides, 20 tracheal swabs were collected from turkeys with respiratory problems. In addition, 23 vaccinated healthy turkey flock Vaccinated turkey flocks with a subtype A of aMPV vaccine were healthy, while the others that were immunized with a subtype B virus vaccine showed respiratory signs	Out of 62,418 (2.9%) of 624 tracheal samples and 18 of 20 tracheal swabs were aMPV positive. Moreover, 1 of 23 healthy and 4 flocks with signs were aMPV positive. Thirty-six samples were positive for subtype B of aMPV	Vero cells, chicken embryo fibroblast cells, and RT-PCR	Ongor et al. (2010)
	Trachea tissues and swabs were taken from 110 non-vaccinated broiler flocks distributed in different geographical regions	Eight out of 110 (7.2%) broiler farms were positive for subtype B of aMPV. Three aMPV isolates were clustered closely with Israel isolate, and the remaining 5 ones were closely related to a vaccine strain from nearby vaccinated turkey farms	RT-PCR	Bayraktar et al. (2018)

Note. AIV = avian influenza virus; aMPV = avian metapneumovirus; IBV = infectious bronchitis virus; MG = *Mycoplasma gallisepticum*; NDV = Newcastle disease virus; ORT = *Ornithobacterium rhinotracheale;* SHS = swollen head syndrome

SUSCEPTIBILITY

Species

Turkeys and chickens are the natural hosts of aMPV subtypes A and B (M. Yu et al., 2019). However, all aMPV subtypes are adapted to Galliformes, particularly turkeys. Subtype C of duck origin is well adapted to ducks; however, chickens and turkeys showed seroconversion and positive virus isolation. It has been investigated that aMPV strains of chicken origin are antigenically closely related to those of turkey origin (Cook et al., 1993). Chickens showed less frequency of aMPV infection, which may be due to

the low shedding capability of this species, as the virus tends to cluster at the temporal and geographical levels (Brown et al., 2019). Chicken flocks are also susceptible to subtype B of aMPV, and they showed seroconversion without signs or shedding of subtypes A and C of turkey and duck lineages. Despite the tropism of subtype C for ducks, chickens could be infected by this subtype (Wei et al., 2013). Turkeys are susceptible and transmit all aMPV subtypes except for subtype C of the duck lineage (S. Sun et al., 2014). They showed clinical signs following an experimental challenge of ducks with subtype C of aMPV (Brown et al., 2019). Antibodies to aMPV have been detected in non-vaccinated, apparently healthy Pekin and Muscovy duck flocks for the first time in Egypt (Nagy et al., 2018). Jardine et al. (2018) isolated subtype C from wild waterfowl (37-44%) in Canada. Recently, Tucciarone et al. (2022) in Italy molecularly detected subtype C of aMPV in a mallard duck and concluded that the short period of the virus infection and transmission reduces the possibility of its detection and increases its significance. In Northern Italy, a mallard duck flock was found to be seropositive for subtype C at slaughter (Legnardi et al., 2021). The virus also was demonstrated in wild geese (Bennett et al., 2005).

In the USA, aMPV originated from wild bird populations and was detected in turkeys. Moreover, pheasants (Gough et al., 2001) and Guinea fowl (Cecchinato et al., 2018) are susceptible to infection and development of swollen head signs. Ostriches were found to be seropositive for aMPV (Cadman et al., 1994). In Brazil, a subtype A of aMPV has been reported in some wild populations of ducks (wood ducks, mandarin ducks, and white-faced whistling ducks), American kestrels, and white-eyed parakeets (Rizotto et al., 2019), but subtype B has been found in rock pigeons, white-faced whistling, and white-cheeked pintails (Felippe et al., 2011). The RNA of subtype C of the virus was detected in black, mallards, wood ducks, geese, blue-winged teals, shovelers, wigeons, sparrows, barn swallows, and starlings in the USA (Bennett et al., 2004; Shin, Njenga, et al., 2000; Shin, Rajashekara, et al., 2000), as well as in mallards, greylag geese, and common gulls in Europe (van Boheemen et al., 2012). Additionally, the RNA of aMPV was found in snow geese, house sparrows, blue-winged teal, earns, and round-billed gulls in Canada (Jardine et al., 2018). Seroconversions against aMPV have been detected in American crows, American coots, Canadian geese, rock pigeons, and cattle egrets in the USA (Turpin et al., 2008). The recent results of aMPV sequencing revealed the presence of 2 new subtypes in a monk parakeet and a great black-backed gull (Canuti et al., 2019). These subtypes may be intermediate between the avian clusters of A, B, C, and D and the human clusters of A and B (Retallack et al., 2019).

Age

All age groups can get infected with aMPV. Broilers are more susceptible to the virus infection than layers and breeders (Tamam et al., 2015). Turkeys are susceptible

for TRT infection at 3 to 12 weeks old. However, 4 to 9 weeks old turkey poults are severely affected. The aMPV tends to affect birds older than 26 days of age (Franzo et al., 2020).

MODE OF INFECTION AND TRANSMISSION

Infection with aMPV is most likely horizontal (airborne) via aerosol or dust particles (Alkahalaf et al., 2002). The virus is highly infectious and characterized by a rapid spread. Direct contact between susceptible birds and infected, contaminated objects is the main route of aMPV transmission (Alkahalaf et al., 2002; Cha et al., 2013). However, the short releasing period of the virus from the infected birds allows for a quick absence of it from the surrounding environment (J. Sun et al., 2014).

Wild and migratory free-living birds are another important means of aMPV transmission (Shin et al., 2002). Bennett et al. (2004) found a 95% nucleotide sequence identity between the strains of aMPV isolated from wild birds and those isolated from turkeys. Wild birds can spread the virus from one region to another. For instance, migratory birds have been incriminated in the early immigration of aMPV from South Africa to European countries (M. Yu et al., 2019; S. Sun et al., 2014). Therefore, free-living birds may act as virus reservoirs for commercial poultry flocks (Jardine et al., 2018). Since migratory birds may have repeated contact with domestic birds in farming regions, a particular correlation has been found between aMPV isolated from wild and domestic birds.

Vertical transmission of subtype C of aMPV has been documented in specific pathogen-free laying turkey hens, as the virus has been isolated following experimental contamination of eggs up to 7 days post-infection (PI) (Cook et al., 2000; Jones et al., 1988). However, this pathway of virus transmission may be short-lived with slight importance (Ganapathy et al., 2007).

Mechanical transmission of aMPV via vectors, including contaminated feeders, drinkers, litter, bedding materials, people, and vehicles, is considered an indirect transmission (Alkahalaf et al., 2002).

PATHOGENESIS

The pathogenesis of aMPV is affected by several factors, including the degrees of macroscopic and microscopic damage, immunity, virus shedding, and clinical outcomes (Cha et al., 2013).

After the respiratory affection of the bird with aMPV, the target tissue of the virus is mainly the upper respiratory tract epithelial layer. Moreover, the virus will likely spread in layers and breeders from the respiratory tract to the reproductive organs.

The attachment of aMPV to the epithelial cells is mediated by the G-protein, while the envelope of the virus fuses with the cell membrane of the host through the F protein. Following that, the virus's genome is released in the host cell cytoplasm with a rapid and multiple propagation or multiplication of the virus (Q. Yu et al., 2013). An attenuated or virulent strain of aPMV replicates in the upper respiratory

tract, followed by a short stage of viremia and persistence of the virus for almost 10 days PI (Van de Zande et al., 1998). In this stage, the virus shows fast dissemination and shedding in the surrounding environment. The clinical signs usually appear to concur with the viral shedding and may be first detected during 2 to 10 days PI, while the severity of the signs is the greatest between 5 to 7 days PI (Choi et al., 2010). Cook (2000) reported on the limited replication of aMPV in the trachea, lung, or other tissues following natural infection.

For routine isolation of aMPV, the samples should be taken from day 1 till 10 PI. However, the viral genomes could be present in the nasal cleft swabs for up to 28 days PI. When layering turkeys with aMPV, the virus can be detected in the respiratory and genital tracts for up to 9 days after infection. It poses a significant challenge in terms of replication and detection. The presence of virus carriers is limited as the viral shedding is very short. Gharaibeh and Shamoun (2012) correlated the presence or absence of aMPV in the respiratory mucosa with the existence or recovery of microscopic lesions, respectively. The histopathological and immunohistochemical results showed that on the second day PI, aMPV could replicate in the turbinates tissues causing serious rhinitis and increased glandular activity. Besides, the virus could induce epithelial damage and decilliation, congestion, and infiltration of mononuclear inflammatory cells and intracytoplasmic eosinophilic inclusions in the ciliated epithelial cells and submucosa. However, on the third and fourth day, PI, catarrhal rhinitis, mucopurulent exudate, exfoliation of the epithelium, and moderate to severe submucosal mononuclear inflammatory cell infiltration could be observed. Temporary lesions were seen in the trachea, with few or no lesions in the conjunctiva and the other organs (Majó et al., 1995).

It was detected that transient immunosuppression of birds may occur during the acute stage of aMPV infection (Timms et al., 1986). Multiplication of the virus in the cilia of the upper respiratory cells reduces the amount of mucoid secretion and consequently enhances bacterial replication (Jirjis et al., 2004). Moreover, the virus could reduce the thymus weight in turkey poults (Timms et al., 1986). The early stage of aMPV infection may inhibit the later efficacy of the turkey hemorrhagic enteritis virus vaccine and induces immune suppression of vaccinated birds (Chary, Rautenschlein, et al., 2002).

PATHOLOGY

The severity of clinical signs, mortality rate, and aMPV lesions is affected by other infectious and non-infectious complications (Figure 1).

The incubation period of aMPV usually ranges from 3-7 days (Jones et al., 1987). Some infected cases show a sudden onset of signs and rapid virus transmission. An acute, highly contagious upper respiratory disease is usually associated with aMPV infection. Affected birds show unilateral and/or bilateral swelling of the infraorbital sinuses, facial edema, and accumulation

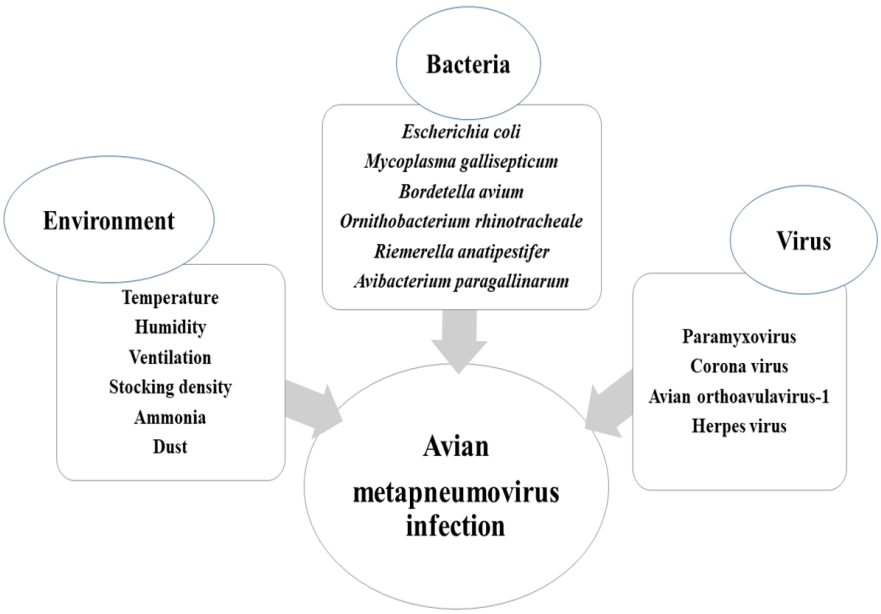

Figure 1. Factors affecting the severity of avian metapneumovirus infection in poultry flocks

of discharge in nostrils, sinuses, and eyes (Jirjis et al., 2002). Snicking, sneezing, periorbital and submandibular edema, and watery frothy to mucopurulent nasal and ocular discharges are the upper respiratory signs that are mostly observed, especially in young birds (Pringle, 1998). Coughing, gasping, dyspnea, and rales can also be detected in the later stages, especially with complications (Jones et al., 1988). General signs, including depression, anorexia, ruffled feathers, and reduced growth and egg performance parameters, are usually associated with respiratory manifestations (Seifi & Boroomand, 2015). Besides the mild respiratory manifestations, aMPV-affected adult layers may exhibit a 10–40% decrease in egg quantity and changes in eggshell quality (Sugiyama et al., 2006). It has been documented that chickens with SHS may show upper respiratory manifestations, cerebral disorientation, torticollis, and opisthotonos (Morley & Thomson, 1984). Concurrent infection with aMPV and IBV may be associated with reduced fertility and orchitis in roosters (Villarreal et al., 2007).

Infection with aMPV is generally characterized by high morbidity (40–100%) and low mortality (1–5%) rates (Sun et al., 2014b) depending on the presence of many factors, including the age of birds, secondary infections, and constitution of the affected flock (Umar et al., 2019). For instance, the mortality rate ranges from 0.5% in adults to 80% in young turkeys (Van de Zande et al., 1998). In cases with concomitant secondary bacterial infection, the mortality rate reached 90% (Awad et al., 2014).

Sudden onset and rapid recovery of aMPV could be observed within 7–10 days

PI, especially in experimentally infected birds with a good constitution and absence of complications (Jones et al., 1988). However, a prolonged disease course for several weeks may be seen in cases reared under poor hygienic conditions and other infections (Zuo et al., 2018). It is assumed that there is no latent or carrier status, and the shedding time of aMPV virus is limited. Without complications, aMPV could be re-isolated from infected birds only for a few days under natural and experimental conditions (Bäyon-Auboyer et al., 1999; Choi et al., 2010). However, in complication, the virus could be detected for up to 7 days following infection.

Differences in replication ability and virulence of aMPV subtypes have been noticed following experimental infections of ducklings, turkeys, and chickens (Aung et al., 2008; dos Santos et al., 2012). For instance, the challenge of domestic chickens with aMPV revealed less severe clinical signs than those in turkeys. Chickens seem vulnerable to experimental but not natural infection with the USA "Colorado" strain or subtype C of aMPV (Cook et al., 1999). Moreover, this subtype in ducks may induce respiratory manifestations and a drop in egg production (Toquin et al., 2006). It is important to note that some aMPV infected flocks did not show any respiratory manifestations, but serological evidence of specific neutralizing antibodies indicates infections in the infected birds (Owoade et al., 2006).

The post-mortem lesions of aMPV revealed the presence of excessive exudates in the respiratory tract, which represented rhinitis, conjunctivitis, sinusitis, laryngitis, and tracheitis. Lower respiratory infections, including airsacculitis, pneumonia, pericarditis, and perihepatitis, could be observed in the presence of concomitant bacterial infection (Zuo et al., 2018). Furthermore, osteomyelitis and subcutaneous head edema may be noticed (Cha et al., 2007; Jirjis et al., 2002).

The histopathological examination of aMPV-affected tissues revealed the presence of necrosis and damage of upper respiratory mucosa, loss of cilia, severe infiltration with inflammatory or lymphoid cells in the submucosa, and substantial tracheitis (Cha et al., 2007; Chary, Rautenschlein, et al., 2002). The virus or the intracytoplasmic inclusion bodies could be seen in the epithelial cells of the respiratory tract following the second or third day of PI.

LABORATORY DIAGNOSIS

The observation of signs and post-mortem lesions of aMPV infection can be considered a preliminary diagnostic step for the diagnosis**.** However, many similar bacterial, viral, and fungal diseases may be confusing. Therefore, the laboratory diagnosis is very important to confirm the aMPV infection.

The aMPV diagnosis relies on virus detection using molecular techniques and serology. Sometimes, isolation of aMPV from chicken tissues is harder than from turkey tissues, possibly due to secondary *E. coli* infection concurrent with SHS. Moreover, other viruses in samples may overcome AMPV propagation during the isolation process on tissue cultures.

Isolation and Identification

The sampling time is crucial, as aMPV is only detected in the upper respiratory organs in the early first week of infection. Sample collections for virus isolations should be done during the early phase of the infection because the virus is present in the sinuses, choanal clefts, and turbinates for a very short time. It is why early sampling in acute infection is commended (Jones et al., 1988). The isolation process of aMPV, a few days of PI, may be difficult because the secondary bacterial infections with the short shedding period of the virus create difficulties in the isolation process (Cook et al., 2001). Swabs from the secretion and tissues of the upper respiratory organs and trachea in the acute phase of aMPV infection are the samples of choice (Cook & Cavanagh, 2002). The virus could be isolated from the trachea, lungs, and sometimes visceral tissues of the infected young turkeys. Successful isolation of the virus from birds in chronic stages of infection is rare. Samples should be kept on ice and immediately transported or frozen for further processing.

Samples could be processed for the conventional isolation techniques. Primary isolation of aMPV could be applied through yolk sac inoculation of 6–8-day-old specific pathogen-free embryonated chicken or turkey eggs (Buys et al., 1989). Positive AMPV samples show stunting and few deaths of the embryos within 7–10 days. However, the virus may need 2 to 9 embryo passages to induce embryonic lesions and deaths.

Moreover, the yolk sac membrane homogenate could be inoculated on a specific tissue culture to detect the viral cytopathic effect (Coswig et al., 2010). However, this method for isolation is laborious, slow, expensive, and needs successive repeated passages to detect the virus. The tracheal organ culture from turkey embryos or turkey poults (Cook & Cavanagh, 2002), chicken embryo fibroblast cells (Coswig et al., 2010), Vero cells (Shin et al., 2002), chick embryo rough cells (Dani et al., 1999), and QT35 quail cells (Coswig et al., 2010) have been used for conventional isolation of aMPV. The virus may take 7 days on the primary passage (Jones et al., 1991), but mostly, cultures should be kept under observation for 11 days with 7 to 8 blind passages (Coswig et al., 2010) to produce ciliostasis and a characteristic cytopathic syncytia formation. Immunofluorescence and staining could be done for confirmation of cryostasis (Bhattacharjee et al., 1994).

Because of the fastidious nature of aMPV, molecular techniques are now applied as alternatives to the traditional isolation methods for rapid virus detection (Mo et al., 2022). Reverse transcription-polymerase chain reaction (RT-PCR) can be used as a highly specific, sensitive, and rapid technique for the detection and subtyping of aMPV from mouth, nose, and tracheal swabs (Marianna et al., 2019; Lemaitre et al., 2018). The primer sequences for the RT-PCR have been designed using targeted M, F, N, and G genes (Bäyon-Auboyer et al., 1999). Isolates of aMPV are molecularly heterogenic. Therefore,

most RT-PCR techniques are subgroup-specific and cannot identify all subgroups (Pedersen et al., 2000). Primers should be directed to the conserved regions of N and G genes to obtain broader specificity and to detect all subtypes (Lwamba et al., 2005). Sequencing techniques can confirm the PCR product identity. Quantitative RT-PCR has also been applied to detect the viral load in a sample (Guionie et al., 2007). Virus sequencing using RT-nested PCR or restriction endonuclease digestion is essential for differentiating between the vaccine and field virus strains (Listorti et al., 2014) and detecting subtypes A and B viruses (Cook & Cavanagh, 2002). The subtype C of aMPV was detected by isolation and RT-PCR up to 6 days PI of 3-weeks-old turkeys (Jirjis et al., 2000).

Other techniques, such as immunohistochemistry staining (Shin et al., 2002), immunofluorescent assay (Cook & Cavanagh, 2002), and *in situ* hybridization (Velayudhan et al., 2005) have also been applied for the detection of aMPV. Although monoclonal antibodies are used to characterize and detect subtypes A and B (Cook et al., 1993), they are laborious, time-consuming, and costly (Cook & Cavanagh, 2002).

Serological Tests

The success of aMPV isolation from turkeys displaying severe chronic symptoms is rare (Naylor & Jones, 1993). It is important to note that the serological response to aMPV infection in chickens is weaker than in turkeys. However, serology is the most common diagnostic tool for the virus, especially in non-vaccinated flocks where seroconversion is a clear indicator of field virus contact (Cook, 2000). Moreover, serology is a common detection method due to difficulties isolating and identifying aMPV.

Some serological tests such as enzyme-linked immunosorbent assay (ELISA) (Ali et al., 2019; Nagy et al., 2018; Zuo et al., 2018) and serum neutralization (SN) (Kapczynski et al., 2008) test is used for serological monitoring and screening of antibodies against aMPV. Commercial ELISA kits containing aMPV-specific monoclonal antibodies have broad specificity and sensitivity for every subtype in various avian species (Aly et al., 1997). Some commercial ELISA kits can detect subtypes A and B of aMPV without differentiation, as both belong to a common serotype (Toquin et al., 1996). However, ELISA kits cannot detect antibodies to aMPV subtype C with subtypes A and B antigens. Choi et al. (2010) suggested that detecting antibodies to aMPV infection in layer chickens using yolk ELISA may be an appropriate substitute for serum. It has been found that there is a close relation between ELISA and other serological tests but subtypes A and B of the virus showed a cross-reaction in the SN test (Cook & Cavanagh, 2002; Jones et al., 1988). The commercial ELISA kit detected aMPV during the first 10 days of PI, while the competitive ELISA started to detect antibodies as early as 5 days of PI (Choi et al., 2010). The competitive ELISA showed specificity and sensitivity of 100 and 98.0%,

respectively, compared to the SN test (Choi et al., 2010).

Additionally, indirect immunofluorescence, virus neutralization, and immunodiffusion tests may be applied to tissue sections to detect specific aMPV antibodies. It is well known that aMPV does not cause hemagglutination of red blood cells. Thus, the hemagglutination-inhibition test cannot be used to detect specific antibodies.

THE IMMUNE RESPONSE AGAINST AMPV INFECTION

Both the humoral and cell-mediated immune response plays a role in aMPV infection. The immune reaction against aMPV in broiler chickens differs from that in turkeys. Experimental infection of broiler chickens with subtypes A and B of aMPV of turkey origin provoked a mild respiratory sign, correlated with the induction of local and systemic virus-neutralizing specific antibodies (Rautenschlein et al., 2011). The neutralizing antibodies began to rise at the peak of signs (6 days PI), while IgG-ELISA titers were high between 24 and 28 days PI (Rautenschlein et al., 2011). The levels of specific IgA-ELISA and neutralizing antibodies in tracheal washes decreased by 10 and 14-days PI, respectively, which may clarify the re-infection with the field AMPV (Rautenschlein et al., 2011). Both subtypes A and B induced up-regulation of the nasal interferon-γ mRNA expression but only subtype A enhanced this expression in the Harderian gland of the eye. Moreover, aMPV-infected broiler chickens showed CD4 and T cells in the Harderian gland, while turkeys showed increased CD8alpha and cells at 6-day PI (Rautenschlein et al., 2011). In the study of Liman and Rautenschlein (2007), the local and systemic humoral and cell-mediated immune reactions following infection of turkeys with an attenuated vaccine strain of a subtype B of aMPV and virulent strains of subtypes A and B were investigated. The results indicated that the neutralizing antibodies were seen in the tracheal washes and the serum 5–7 days PI and then declined, while the level of ELISA antibody appeared from 14–28 days PI. Moreover, subtypes A and B of aMPV infection induced humoral and cell-mediated immunity in comparison with subtype C infection.

PREVENTION AND CONTROL

Biosecurity measures and vaccination programs play an important role in preventing deaths, drops in egg production, and changes in egg quality resulting from aMPV infection. Vaccines succeeded in providing cellular and humoral protective immune responses against the virus.

Biosecurity

Good biosecurity measures should be adopted in the farms to prevent and control aMPV. Prohibit multiage sites as they are always at risk. It is important to decrease stress on the respiratory tract that may predispose birds to secondary bacterial infections. For example, controlling air

quality, ventilation, heating, and misting of dry litter is very important. Control measures should be carried out after aMPV infection, including depopulation, hygienic disposal of carcasses, washing, a reasonable period between batches, marketing restrictions, and good hygienic measures.

Vaccination

Inactivated Vaccines. Oil emulsion or water adjuvant inactivated aMPV vaccines containing subtypes A and B have been used. These vaccines provoke long-term protection of aMPV infection, replication, excretion, reduction of clinical picture severity, and prevention of egg quantity and quality changes (Hess et al., 2004; Tamam et al., 2015). They are given before laying in breeder and layer turkeys (Van de Zande et al., 2000). Intramuscular vaccination of breeder turkeys with aMPV inactivated vaccine at 30 weeks old after priming with a live attenuated vaccine at 7 days of age prevented the drop in egg production at 38 weeks of age. Also, broiler breeders and layers may be given a live aMPV vaccine at 10 to 12 weeks, followed by a killed vaccine at 16 to 20 weeks. Turkey breeders could be primed with a coarse spray of a live aMPV vaccine at 2 weeks old, followed by inoculation of the inactivated vaccine at 22 weeks old (Cook et al., 1996). However, the usual program in turkey's vaccination includes the administration of an inactivated aMPV vaccine 4–6 weeks of age following the last vaccination with a live vaccine up to 28 weeks of age but avoid the last 4 weeks before laying. Turkey poults could be vaccinated with an inactivated aMPV vaccine with promising results. In the Egyptian study of Tamam et al. (2015), the findings indicated that the locally prepared aMPV inactivated vaccine provoked significant high humoral and cell-mediated immune responses as well as protection rates up to 100% in vaccinated and challenged 3-week-old-turkey poults.

Live Attenuated Vaccines. Living attenuated vaccines against TRT infection were first described in Europe in the early 1990s. These vaccines are given for growing turkey poults to prevent the development of clinical respiratory signs in young and to prime inactivated vaccines in future breeders. Live vaccines could successfully control TRT infection (Cook, 2000). Live attenuated TRT vaccines have been derived from aMPV subgroups A and B in Europe and from a subgroup C in the USA. According to the dominant subtype of aMPV in the region, subtype B and A live vaccines may maximize protection. A single dose of live vaccine can enhance the poor immune response when compared with infection with a virulent field aMPV. These vaccines have been applied to provide excellent local and cell-mediated immune responses in the absence of maternal-derived antibodies. Accordingly, turkeys that received live attenuated aMPV vaccines showed activation of CD8+ and CD4+ T lymphocytes in both the Harderian glands of eyes and tracheal mucosa as well as production of immunoglobulin (Ig) A. Whereas, there is a minor role of humoral immune response in the protection of turkey

poults against TRT infection (Jones et al., 1992; Naylor, Worthington, et al., 1997). The development of humoral immune response and, consequently, the efficacy of the vaccination process against TRT might be affected by the maternal-delivered antibodies (Rubbenstroth & Rautenschlein, 2009; Śmiałek et al., 2016, 2021). It has been documented that the titers of serum antibodies rapidly increased during the first 7 days after immunization of turkeys having low maternal antibodies (Śmiałek et al., 2015). Some interference between maternal-derived antibodies and living TRT vaccines may occur in young turkeys with higher maternal immunity. Turkeys with maternally derived antibodies displayed low virus-specific interferon (IFN)-gamma-secreting cells after vaccination with the TRT vaccine (Śmiałek et al., 2020).

Different vaccination protocols have been applied for the live TRT vaccine. The first one is the vaccination of turkeys one day till 7 days old, while the second protocol may be applied around 3 to 6 weeks old or after 6 weeks old. Repeated vaccinations may be important to induce a prolonged immune response and to prevent interference with maternal immunity to the hemorrhagic enteritis virus vaccine that is given concurrently with the TRT vaccine.

Birds can receive live vaccines against aMPV in the form of a spray, drinking water, or eye drops. Spray or drinking water routes gave satisfactory protection against the development of the clinical picture compared with the ocular-oral route (Ganapathy et al., 2010).

Subtypes A and B of aMPV could be detected in immunized and non-immunized birds. Moreover, a vaccine containing a subtype A strain may become virulent and distributed in turkey flocks inducing typical TRT signs (Lupini et al., 2011). Some reports showed that live vaccines against TRT may return to their virulence after repetitive passages in susceptible hosts (Catelli et al., 2006; Lupini et al., 2011). Besides, mutation of the field aMPV in response to sustained vaccination is evident (Catelli et al., 2010). Some variant strains of aMPV showed an increased capability to resist the immunity provoked by the used vaccines (Catelli et al., 2010). Formulating new vaccines that do not revert to virulence has gained a great effort. Accordingly, new methyltransferase-defective live attenuated aMPV vaccines have been developed to reduce the viral return to virulence and stop the emergence of field strains that may disturb the vaccine immunity (Y. Zhang et al., 2016).

The cross-protection between the live aMPV vaccine and the subgroups A or B of the challenging virus has been detected (Velayudhan et al., 2005). Vaccines of aMPV containing subtypes A and B gave some protection against the challenge with subtype C but not vice versa (Jones, 2010). Though the presence of good cross-protection between subtypes A and B in the live aMPV vaccines (Cook et al., 1995; Toquin et al., 1996), vaccinated birds still show the disease in different countries with high stocking density (Catelli et al., 2010; Chacón et al., 2011). Unfortunately, some studies in Brazil and Italy revealed that the

current vaccines are not completely efficient against the novel isolates of aMPV (Banet-Noach et al., 2009). In Brazil, dos Santos et al. (2012) found full heterologous protection induced by a live vaccine containing subtypes A and B of aMPV, and they expected that subtype A replicated less efficiently than the subtype B isolates. Moreover, the field viruses may multiply in the immunized birds and shed into the environment.

Infections with aMPV are caused in different ways, including infection by a field subtype that is not involved in the used vaccine (Banet-Noach et al., 2005, 2009), genetic variations between the vaccine and the field strains causing immune evasion (Banet-Noach et al., 2009; Catelli et al., 2010), or insufficient vaccine dose that leads to returning the virus to its virulence (Catelli et al., 2006). Some reports showed a genetic deviation between the field aMPV of subtype B origin and the vaccine strain, which resulted in the absence of homologous protection (Banet-Noach et al., 2009; Catelli et al., 2010). The field virus could overcome the immune response provoked by the vaccine-virus strain owing to the variation in the amino acids between both viruses in the G gene products and SH, respectively. Although a live aMPV vaccine containing subtype B gave 100% protection against clinical disease, one of 10 challenged chicks was positive by virus isolation or RT-PCR after challenge at 21 or 49 days of age (Ganapathy & Jones, 2007).

Some live vaccines may contain aMPV and other viruses such as IBV or NDV (bi or tri-valent vaccine). The interaction between these viruses in one vaccine has been studied. Cook et al. (2001) found that the IBV vaccine interfered with the replication of aMPV vaccine due to the competition of both viruses for the respiratory cell receptors and consequently causing the damage of cells and the reduction of aMPV replication. To avoid the problem of vaccine interference in chickens, live aMPV vaccines should be given at different intervals from IBV vaccines. Besides, dual vaccination against aMPV and NDV induced temporary suppression of aMPV replication, but the virus was found for up to 24 days post-immunization of chickens (Ganapathy et al., 2005). Therefore, immunization against NDV and aMPV did not affect their efficacies (Jones, 2010). Despite other reports showing that the produced protection by live vaccines of IBV, NDV, and aMPV did not cooperate when the vaccines were given concurrently in dual or triple mixtures, the humoral immune response to aMPV may be insufficient (Awad et al., 2015). In addition, giving live vaccines containing subtype B of aMPV, NDV, alongside the classical and variant strains of IBV vaccines, to maternal antibodies-positive day-old broiler chicks did not restrict any protection produced against these viruses (Ball et al., 2019).

Recombinant and DNA Vaccines. Recombinant and DNA vaccine, which encodes fusion glycoprotein (F) of aMPV and a fowlpox virus, has been produced and successfully investigated in turkeys

(Qingzhong et al., 1994). Reduction of clinical manifestations, post-mortem lesions, and the virus multiplication in the respiratory system was reported after vaccination and challenge with the virulent aMPV. The same results were also observed after the vaccination of turkeys with DNA vaccines containing N protein (Kapczynski & Sellers, 2003). Hu et al. (2011) demonstrated that a single dose vaccination of turkeys with a recombinant NDV, encoding glycoprotein G of subtype C of aMPV, provoked moderate immune response and partial protection against aMPV but satisfactory immune response to NDV. The vector vaccine containing the F and G genes of subtype C of aMPV and NDV has been recently produced in turkeys, and the vaccinated birds showed specific antibodies against both viruses and resisted the challenges with both virulent viruses (Hu et al., 2017). Furthermore, the DNA vaccine with bacterial vectors encoding the F gene revealed significant prevention from subtype B of aMPV infection in chickens (Madbouly et al., 2014) and in turkeys (Kapczynski & Sellers, 2003). A recombinant vaccine containing subtype C of aMPV and expressing M and N glycoproteins proteins (Chary et al., 2005) and virosome (Kapczynski, 2004) showed a good immune response in vaccinated turkeys.

In addition, *in ovo*, vaccination with DNA or subunit aMPV vaccines has been shown to be an effective and practical method for protecting chicks and turkey poults against early infection (Jones, 2010).

CONCLUSION

The aMPV is an important infection that affects a wide range of domestic and wild poultry species with different ages. Such infections are widely distributed and recorded in many continents and countries worldwide. Adverse effects on the growth and egg-laying performance parameters have usually been recorded in different aMPV infections, particularly environmental and infectious complications. Despite the development of inactivated, live, and recombinant aMPV vaccine, the different subtypes of the virus are still circulating in the commercial broiler and turkey production system. Therefore, regular surveillance and monitoring of the circulating aMPV and adotping strict biosecurity measures are essential for preventing such infection. Besides, preventing predisposing or concurrent infections is a must to avoid increasing the incidence and severity of aMPV infection.

ACKNOWLEDGEMENTS

The author is thankful to the Faculty of Veterinary Medicine, Cairo University, Egypt, for providing the necessary facilities for this review.

REFERENCES

Abdel-Azeem, A.-A. S., Franzo, G., Zotte, A. D., Drigo, M., Catelli, E., Lupini, C., Martini, M., & Cecchinato, M. (2014). First evidence of avian metapneumovirus subtype A infection in turkeys in Egypt. *Tropical Animal Health and Production*, *46*, 1093–1097. https://doi.org/10.1007/s11250-014-0591-8

Abdelmoez, N., Shawky, M., Abdelhady, H., Lebdah, M., & Salama, S. (2019). Isolation and identification of some possible causative agents of swollen head syndrome (SHS) in broiler chickens in Egypt. *Slovenian Veterinary Research*, *56*(22-Suppl), 781–788. https://doi.org/10.26873/SVR-819-2019

Al-Hasan, B. A., Alhatami, A. O., Abdulwahab, H. M., Bustani, G. S., & Alkuwaity, E. A. W. (2021). The first isolation and detection of *Ornithobacterium rhinotracheale* from swollen head syndrome-infected broiler flocks in Iraq. *Veterinary World*, *14*(9), 2346–2355. https://doi.org/10.14202/vetworld.2021.2346-2355

Al-Hasan, B. A., Alhatami, A. O., Abdulwahab, H. M., Bustani, G. S., Hameed, M. A., & Jawad, A. H. (2022). First report of *Avian metapneumovirus* type B in Iraqi broiler flocks with swollen head syndrome. *Veterinary World*, *15*(1), 16–21. https://doi.org/10.14202/vetworld.2022.16-21

Ali, M. Z., Park, J.-E., & Shin, H.-J. (2019). Serological survey of avian metapneumovirus infection in chickens in Bangladesh. *Journal of Applied Poultry Research*, *28*(4), 1330–1334. https://doi.org/10.3382/japr/pfz050

Alkahalaf, A. N., Ward, L. A., Dearth, R. N., & Saif, Y. M. (2002). Pathogenicity, transmissibility, and tissue distribution of avian pneumovirus in turkey poults. *Avian Diseases*, *46*(3), 650–659. https://doi.org/10.1637/0005-2086(2002)046[0650:PTATDO]2.0.CO;2

Aly, M. M., El-Zaher, A. A., & Amin, A. (1997). Studies on swollen head syndrome in Egypt 1. Serological survey and comparison between two ELISA kits for detection of antibody. *Veterinary Medical Journal Giza*, *45*(2), 251-258.

Arafa, A.-S., Mady, W., Hussein, A., Tamam, S., & Madbouly, H. (2015). Molecular characterization of vaccine-derived mutants of avian metapneumoviruses isolated from turkeys in Egypt. *American Journal of Virology*, *4*(1), 1–11. https://doi.org/10.3844/ajvsp.2015.1.11

Aung, Y. H., Liman, M., Neumann, U., & Rautenschlein, S. (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 Pathology*, *37*(1), 65–74. https://doi.org/10.1080/03079450701802222

Awad, F., Baylis, M., Jones, R. C., & Ganapathy, K. (2014). Evaluation of flinders technology associates cards for storage and molecular detection of avian metapneumoviruses. *Avian Pathology*, *43*(2), 125–129. https://doi.org/10.1080/03079457.2014.885114

Awad, F., Forrester, A., Baylis, M., Lemiere, S., Jones, R., & Ganapathy, K. (2015). Immune responses and interactions following simultaneous application of live Newcastle disease, infectious bronchitis and avian metapneumovirus vaccines in specific-pathogen-free chicks. *Research in Veterinary Science*, *98*, 127–133. https://doi.org/10.1016/j.rvsc.2014.11.004

Ball, C., Forrester, A., Herrmann, A., Lemiere, S., & Ganapathy, K. (2019). Comparative protective immunity provided by live vaccines of Newcastle disease virus or avian metapneumovirus when co-administered alongside classical and variant strains of infectious bronchitis virus in day-old broiler chicks. *Vaccine*, *37*(52), 7566–7575. https://doi.org/10.1016/j.vaccine.2019.09.081

Banet-Noach, C., Simanov, L., & Perk, S. (2005). Characterization of Israeli avian metapneumovirus strains in turkeys and chickens. *Avian Pathology*, *34*(3), 220–226. https://doi.org/10.1080/03079450500112625

Banet-Noach, C., Simanov, L., Laham-Karam, N., Perk, S., & Bacharach, E. (2009). Longitudinal survey of avian metapneumoviruses in poultry in Israel: Infiltration of field strains into vaccinated flocks. *Avian Diseases*, *53*(2), 184–189. https://doi.org/10.1637/8466-090408-reg.1

Bäyon-Auboyer, M. H., Arnauld, C., Toquin, D., & 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. *Journal of General Virology*, *81*(11), 2723–2733. https://doi.org/10.1099/0022-1317-81-11-2723

Bäyon-Auboyer, M. H., Jestin, V., Toquin, D., Cherbonnel, M., & Eterradossi, N. (1999). Comparison of F-, G- and N-based RT-PCR protocols with conventional virological procedures for the detection and typing of turkey rhinotracheitis virus. *Archives of Virology*, *144*, 1091–1109. https://doi.org/10.1007/s007050050572

Bayraktar, E., Umar, S., Yilmaz, A., Turan, N., Franzo, G., Tucciarone, C. M., Cecchinato. M., Cakan, B., Iqbal, M., & Yilmaz, H. (2018). First molecular characterisation of avian metapneumovirus (aMPV) in Turkish broiler flocks. *Avian Diseases*, *62*(4), 425–430. https://doi.org/10.1637/11915-061818-resnote.1

Bennett, R. S., LaRue, R., Shaw, D., Yu, Q., Nagaraja, K. V., Halvorson, D. A., & Njenga, M. K. (2005). A wild goose metapneumovirus containing a large attachment glycoprotein is avirulent but immunoprotective in domestic turkeys. *Journal of Virology*, *79*(23), 14834-14842. https://doi.org/10.1128/jvi.79.23.14834-14842.2005

Bennett, R. S., Nezworski, J., Velayudhan, B. T., Nagaraja, K. V., Zeman, D. H., Dyer, N., Graham, T., Lauer, D. C., Njenga, M. K., & Halvorson, D. A. (2004). Evidence of avian pneumovirus spread beyond Minnesota among wild and domestic birds in central North America. *Avian Diseases*, *48*(4), 902–908. https://doi.org/10.1637/7208-051804r

Bhattacharjee, P. S., Naylor, C. J., & Jones, R. C. (1994). A simple method for immunofluorescence staining of tracheal organ cultures for the rapid identification of infectious bronchitis virus. *Avian Pathology*, *23*(3), 471–480. https://doi.org/10.1080/03079459408419017

Brown, P. A., Allée, C., Courtillon, C., Szerman, N., Lemaitre, E., Toquin, D., Mangart, J. M. M., Amelot, M., & Eterradossi, N. (2019). Host specificity of avian metapneumoviruses. *Avian Pathology*, *48*(4), 311–318. https://doi.org/10.1080/03079457.2019.1584390

Buys, S. B., du Preez, J. H., & Els, H. J. (1989). The isolation and attenuation of a virus causing rhinotracheitis in turkeys in South Africa. *Onderstepoort Journal of Veterinary Research*, *56*(2), 87–98.

Cadman, H. F., Kelly, P. J., Zhou, R., Davelaar, F., & Mason, P. R. (1994). A serosurvey using enzyme-linked immunosorbent assay for antibodies against poultry pathogens in ostriches (*Struthio camelus*) from Zimbabwe. *Avian Diseases*, *38*(3), 621–625. https://doi.org/10.2307/1592088

Canuti, M., Kroyer, A. N. K., Ojkic, D., Whitney, H. G., Robertson, G. J., & Lang, A. S. (2019). Discovery and characterization of novel RNA viruses in aquatic North American wild birds. *Viruses*, *11*(9), 768. https://doi.org/10.3390/v11090768

Catelli, E., Cecchinato, M., Savage, C. E., Jones, R. C., Naylor, C. J. (2006). Demonstration of loss of attenuation and extended field persistence of a live avian metapneumovirus vaccine. *Vaccine*, *24*(42-43), 6476–6482. https://doi.org/10.1016/j.vaccine.2006.06.076

Catelli, E., Lupini, C., Cecchinato, M., Ricchizzi, E., Brown, P., & Naylor, C. J. (2010). Field avian *Metapneumovirus* evolution avoiding vaccine induced immunity. *Vaccine*, *28*(4), 916– 921. https://doi.org/10.1016/j.vaccine.2009.10.149

Cecchinato, M., Lupini, C., Silveira, F., Listorti, V., Mescolini, G., Morandini, E., Franzo, G., & Catelli, E. (2018). Molecular characterization of avian metapneumovirus from Guinea fowls (*Numida meleagridis*). *Pakistan Veterinary Journal, 38*(4), 419–423. https://doi.org/10.29261/pakvetj/2018.088

Cha, R. M., Khatri, M., & Sharma, J. M. (2007). B-cell infiltration in the respiratory mucosa of turkeys exposed to subtype C avian metapneumovirus. *Avian Diseases*, *51*(3), 764-770. https://doi.org/10.1637/0005-2086(2007)51[764:biitrm]2.0.co;2

Cha, R. M., Yu, Q., & Zsak, L. (2013). The pathogenicity of avian metapneumovirus subtype C wild bird isolates in domestic turkeys. *Virology Journal*, *10*, 38. https://doi.org/10.1186/1743-422x-10-38

Chaboki, P. M., Ghalyanchilangeroudi, A., Karimi, V., Abdollahi, H., Maghsoudloo, H., Hosseini, H., Farahahni, R. K., Ghafouri, S. A., Falah, M. H., Rezaee, H., Jabbarifakhr, M., & Mousavi, F. S. (2018). Prevalence of avian metapneumovirus subtype B in live bird market in Gilan province, Iran. *Veterinary Research Forum*, *9*(1), 93–97.

Chacón, J. L., Brandão, P. E., Buim, M., Villarreal, L., & Ferreira A. J. (2007). Detection by reverse transcriptase-polymerase chain reaction and molecular characterization of subtype B avian metapneumovirus isolated in Brazil. *Avian Pathology*, *36*(5), 383–387. https://doi.org/10.1080/03079450701589142

Chacón, J. L., Mizuma, M., Vejarano, M. P., Toquín, D., Eterradossi, N., Patnayak, D. P., Goyal, S. M., & Ferreira, A. J. (2011). Avian metapneumovirus subtypes circulating in Brazilian vaccinated and non-vaccinated chicken and turkey farms. *Avian Diseases*, *55*(1), 82–89. https://doi.org/10.1637/9501-081310-reg.1

Chary, P., Njenga, M. K., & Sharma, J. M. (2005). Protection by recombinant viral proteins against a respiratory challenge with virulent avian metapneumovirus. *Veterinary Immunology and Immunopathology*, *108*(3-4), 427–432. https://doi.org/10.1016/j.vetimm.2005.06.012

Chary, P., Rautenschlein, S., & Sharma, J. M. (2002). Reduced efficacy of hemorrhagic enteritis virus vaccine in turkeys exposed to avian pneumovirus. *Avian Diseases*, *46*(2), 353–359. https://doi.org/10.1637/0005-2086(2002)046[0353:reohev]2.0.co;2

Chary, P., Rautenschlein, S., Njenga, M. K., & Sharma, J. M. (2002). Pathogenic and immunosuppressive effects of avian pneumovirus in turkeys. *Avian Diseases*, *46*(1), 153– 161. https://doi.org/10.1637/0005-2086(2002)046[0153:paieoa]2.0.co;2

Choi, K. S., Kim, J. W., Lee, E. K., Jeon, W. J., Park, M. J., Lyoo, Y. N., & Kwon, J. H. (2010). Development of competitive ELISA for detection of avian metapneumovirus antibodies in chicken. *Journal of Bacteriology and Virology*, *40*(3), 131–143. http://doi.org/10.4167/jbv.2010.40.3.131

Cook, J. K. A. (2000). Avian pneumovirus infections of turkeys and chickens. *The Veterinary Journal*, *160*(2), 118–125. https://doi.org/10.1053/tvjl.2000.0486

Cook, J. K. A., & Cavanagh, D. (2002). Detection and differentiation of avian pneumoviruses (metapneumoviruses). *Avian Pathology*, *31*(2), 117–132. https://doi.org/10.1080/03079450120118603

Cook, J. K. A., Chesher, J., Orthel, F., Woods, M. A., Orbell, S. J., Baxendale, W., & Huggins, M. B. (2000). Avian pneumovirus infection of laying hens: Experimental studies. *Avian Pathology*, *29*(6), 545–556. https://doi.org/10.1080/03079450020016788

Cook, J. K. A., Huggins, M. B., Orbell, S. J., & Senne, D. A. (1999). Preliminary antigenic characterization of an avian pneumovirus isolated from commercial turkeys in Colorado, USA. *Avian Pathology*, *28*(6), 607–617. https://doi.org/10.1080/03079459994407

Cook, J. K. A., Huggins, M. B., Orbell, S. J., Mawditt, K., & Cavanagh, D. (2001). Infectious bronchitis virus vaccine interferes with the replication of avian pneumovirus vaccine in domestic fowl. *Avian Pathology*, *30*(3), 233–242. https://doi.org/10.1080/03079450120054640

Cook, J. K. A., Huggins, M. B., Wood, M. A., Orbell, S. J., & Mockett, A. P. A. (1995). Protection provided by a commercially available vaccine against different strains of turkey rhinotracheitis virus. *Veterinary Record*, *136*(15), 392–393. https://doi.org/10.1136/vr.136.15.392

Cook, J. K. A., Orthel, F., Orbell, S., Woods, M. A., & Huggins, M. B. (1996). An experimental turkey rhinotracheitis (TRT) infection in breeding turkeys and the prevention of its clinical effects using live-attenuated and inactivated TRT vaccines. *Avian Pathology*, *25*(2), 231–243. https://doi.org/10.1080/03079459608419138

Cook, J. K. A., Jones, B. V., Ellis, M. M., Jing, L., & Cavanagh, D. (1993). Antigenic differentiation of strains of turkey rhinotracheitis virus using monoclonal antibodies. *Avian*

Pathology, *22*(2), 257–273. https://doi.org/10.1080/03079459308418919

Coswig, L. T., dos Santos, M. B., Hafez, H. M., Ferreira, H. L., & Arns, C. W. (2010). Propagation of avian metapneumovirus subtypes A and B using chicken embryo related and other cell systems. *Journal of Virological Methods*, *167*(1), 1–4. https://doi.org/10.1016/j.jviromet.2010.02.018

Croville, G., Foret, C., Heuillard, P., Senet, A., Delpont, M., Mouahid, M., Ducatez, M., Kichou, F., & Guerin, J. (2018). Disclosing respiratory co-infections: A broad-range panel assay for avian respiratory pathogens on a nanofluidic PCR platform. *Avian Pathology*, *47*(3), 253–260. https://doi.org/10.1080/03079457.2018.1430891

Dani, M. A. C., Arns, C. W., & Durigon, E. L. (1999). Molecular characterization of Brazilian avian pneumovirus isolates using reverse transcription-polymerase chain reaction, restriction endonuclease analysis and sequencing of a G gene fragment. *Avian Pathology*, *28*(5), 473–476. https://doi.org/10.1080/03079459994498

Darebaghi, A., Chashmi, S. H. E., Kafshdozan, K., & Hosseini, H. (2021). Molecular detection of avian metapneumovirus in Semnan broiler farms. *Iranian Journal of Veterinary Medicine*, *15*(1), 27–34. https://doi.org/10.22059/ijvm.2020.302556.1005087

dos Santos, M. B, Martini M.C., Ferreira, H. L., da Silva, L. H., Fellipe, P. A., Spilki, F. R., & Arns, C.W. (2012). Brazilian avian metapneumovirus subtypes A and B: Experimental infection of broilers and evaluation of vaccine efficacy. *Pesquisa Veterinária Brasileira*, *32*(12), 1257–1262. https://doi.org/10.1590/S0100-736X2012001200008

Felippe, P. A., Silva, L. H., Santos, M. B., Sakata, S. T., & Arns, C. W. (2011). Detection of and phylogenetic studies with avian metapneumovirus recovered from feral pigeons and wild birds in Brazil. *Avian Pathology*, *40*(5), 445–452. https://doi.org/10.1080/03079457.2011.596812

Franzo, G., Legnardi, M., Mescolini, G., Tucciarone, C. M., Lupini, C., Quaglia, G., Catelli, E., & Cecchinato, M. (2020). Avian Metapneumovirus subtype B around Europe: A phylodynamic reconstruction. *Veterinary Research*, *51*, 88. https://doi.org/10.1186%2Fs13567-020-00817-6

Franzo, G., Tucciarone, C. M., Enache, M., Bejan, V., Ramon, G., Koutoulis, K. C., & Cecchinato, M. (2017). First report of avian metapneumovirus subtype B field strain in a Romanian broiler flock during an outbreak of respiratory disease. *Avian Diseases*, *61*(2), 250–254. https://doi.org/10.1637/11557-121216-resnote.1

Ganapathy, K., & Jones R. C. (2007). Vaccination of chicks with live attenuated subtype B avian metapneumovirus vaccines: Protection against challenge and immune responses can be unrelated to vaccine dose. *Avian Diseases*, *51*(3), 733–737. https://doi.org/10.1637/0005-2086(2007)51[733:vocwla]2.0.co;2

Ganapathy, K., Bufton, A., Pearson, A., Lemiere, S., & Jones, R. C. (2010). Vaccination of commercial broiler chicks against avian metapneumovirus infection: A comparison of drinking water, spray and oculo-oral delivery methods. *Vaccine*, *28*(23), 3944–3948. https://doi.org/10.1016/j.vaccine.2010.03.065

Ganapathy, K., Cargill, P., Montiel, E., & Jones, R. C. (2005). Interaction between live avian pneumovirus and Newcastle disease virus vaccines in specific pathogen free chickens. *Avian Pathology*, *34*(4), 297–302. https://doi.org/10.1080/03079450500178824

Ganapathy, K., Cox, W. J., Gough, R. E., Cargill, P., Montiel, E., & Jones, R. C. (2007). Protection in specific pathogen free chickens with live avian metapneumovirus and Newcastle disease virus vaccines applied singly or in combination. *Avian Pathology*, *36*(4), 313–317. https://doi.org/10.1080/03079450701460781

Gharaibeh, S. M., & Algharaibeh, G. R. (2007). Serological and molecular detection of avian pneumovirus in chickens with respiratory disease in Jordan. *Poultry Science*, *86*(8), 1677–1681. https://doi.org/10.1093/ps/86.8.1677

Gharaibeh, S., & Shamoun, M. (2012). Avian metapneumovirus subtype B experimental infection and tissue distribution in chickens, sparrows, and pigeons. *Veterinary Pathology*, *49*(4), 704–709. https://doi.org/10.1177/0300985811402845

Giraud, P., Bennejean, G., Guittet, M., & Toquin, D. (1986). Turkey rhinotracheitis in France: Preliminary investigations on a ciliostatic virus. *Veterinary Record*, *119*(24), 606–607.

Gough, R. E., Drury, S. E., Aldous, E. W., & Laing, P. (2001). Isolation and identification of avian pneumovirus from pheasants. *Veterinary Record*, *149*(10), 312.

Govindarajan, D., & Samal, S. K. (2004). Sequence analysis of the large polymerase (L) protein of the US strain of avian metapneumovirus indicates a close resemblance to that of the human metapneumovirus. *Virus Research*, *105*(1), 59–66. https://doi.org/10.1016/j.virusres.2004.04.014

Graziosi, G., Mescolini, G., Silveira, F., Lupini, C., Tucciarone, C. M., Franzo, G., Cecchinato, M., Legnardi, M., Gobbo, F., Terregino, C., & Catelli, E. (2022). First detection of avian metapneumovirus subtype C Eurasian lineage in a Eurasian wigeon (*Mareca penelope*) wintering in Northeastern Italy: An additional hint on the role of migrating birds in the viral epidemiology. *Avian Pathology*, *51*(3), 283–290. https://doi.org/10.1080/03079457.2022.2051429

Guionie, O., Toquin, D., Sellal, E., Bouley, S., Zwingelstein, F., Allee, C., Bougeard, S., Lemiere, S., & Eterradossi, N. (2007). Laboratory evaluation of a quantitative real-time reverse transcription PCR assay for the detection and identification of the four subgroups of avian metapneumovirus. *Journal of Virological Methods*, *139*(2), 150–158. https://doi.org/10.1016/j.jviromet.2006.09.022

Hess, M., Huggins, M. B., & Heincz, U. (2004). Hatchability, serology and virus excretion following *in ovo* vaccination of chickens with an avian metapneumovirus vaccine. *Avian Pathology*, *33*(6), 576–580. https://doi.org/10.1080/03079450400013220

Hosseini, H., & Ghalyanchi-Langeroudi, A. (2012). Detection and molecular characterization of avian metapneumovirus in Iran: The first report. *Iranian Journal of Virology*, *6*(2), 26–31. https://doi.org/10.21859/isv.6.2.26

Hosseini, H., Ghalyanchilangeroudi, A., & Mousavi, F. S. (2017). Characterization of Iranian avian metapneumovirus based on fusion gene (F). *Iranian Journal of Virology*, *11*(1), 39-43.

Hu, H., Roth J. P., Zsak, L., & Yu, Q. (2017). Engineered Newcastle disease virus expressing the F and G proteins of AMPV-C confers protection against challenges in turkeys. *Scientific Reports*, *7*, 4025. https://doi.org/10.1038/s41598-017-04267-7

Hu, H., Roth, J. P., Estevez, C. N., Zsak, L., Liu, B., & Yu, Q. (2011). Generation and evaluation of a recombinant Newcastle disease virus expressing the glycoprotein (G) of avian metapneumovirus subgroup C as a bivalent vaccine in turkeys. *Vaccine*, *29*(47), 8624–8633. https://doi.org/10.1016/j.vaccine.2011.09.007

Jardine, C. M., Parmley, E. J., Buchanan, T., Nituch, L., & Ojkic, D. (2018). Avian metapneumovirus subtype C in wild waterfowl in Ontario, Canada. *Transboundary and Emerging Diseases*, *65*(4), 1098–1102. https://doi.org/10.1111/tbed.12832

Jirjis, F. F., Noll, S. L., Halvorson, D. A., Nagaraja, K. V., & Shaw, D. P. (2002). Pathogenesis of avian pneumovirus infection in turkeys. *Veterinary Pathology*, *39*(3), 300–310. https://doi.org/10.1354/vp.39-3-300

Jirjis, F. F., Noll, S. L., Halvorson, D. A., Nagaraja, K. V., Martin, F., & Shaw, D. P. (2004). Effects of bacterial coinfection on the pathogenesis of avian pneumovirus infection in turkeys. *Avian Diseases*, *48*(1), 34–49. https://doi.org/10.1637/7017

Jirjis, F. F., Noll, S. L., Halvorson, D. A., Nagaraja, K. V., Townsend, E. L., Sheikh, A. M., & Shaw, D. P. (2000). Avian pneumovirus infection in Minnesota turkeys: Experimental reproduction of the disease. *Avian Diseases*, *44*(1), 222–226. https://doi.org/10.2307/1592530

Jones, R. C. (2010). Viral respiratory diseases (ILT, aMPV infections, IB): Are they ever under control? *British Poultry Science*, *51*(1), 1–11. https://doi.org/10.1080/00071660903541378

Jones, R. C., Baxter-Jones, C., Savage, C. E., Kelly, D. F., & Wilding, G. P. (1987). Experimental infection of chickens with a ciliostatic agent isolated from turkeys with rhinotracheitis. *Veterinary Record*, *120*(13), 301–302. https://doi.org/10.1136/vr.120.13.301

Jones, R. C., Naylor, C. J., Al-Afaleq, A., Worthington, K. J., & Jones, R. (1992). Effect of cyclophosphamide immunosuppression on the immunity of turkeys to viral rhinotracheitis. *Research in Veterinary Science*, *53*(1), 38–41. https://doi.org/10.1016/0034-5288(92)90081-c

Jones, R. C., Naylor, C. J., Bradbury, J. M., Savage, C. E., Worthington, K., & Williams, R. A. (1991). Isolation of a turkey rhinotracheitis-like virus from broiler breeder chickens in England. *Veterinary Record*, *129*(23), 509–510.

Jones, R. C., Williams, R. A., Baxter-Jones, C., Savage, C. E., & Wilding, G. P. (1988). Experimental infection of laying turkeys with rhinotracheitis virus: Distribution of virus in the tissues and serological response. *Avian Pathology*, *17*(4), 841–850. https://doi.org/10.1080/03079458808436506

Kapczynski, D. R. (2004). Development of a virosome vaccine against avian metapneumovirus subtype C for protection in turkeys. *Avian Diseases*, *48*(2), 332–343. https://doi.org/10.1637/7115

Kapczynski, D. R., & Sellers, H. S. (2003). Immunization of turkeys with a DNA vaccine expressing either the F or N gene of avian metapneumovirus. *Avian Diseases*, *47*(4), 1376–1383. https://doi.org/10.1637/7033

Kapczynski, D. R., Perkins, L. L., & Sellers, H. S. (2008). Mucosal vaccination with formalin inactivated avian metapneumovirus subtype C does not protect turkeys following intranasal challenge. *Avian Diseases*, *52*(1), 28–33. https://doi.org/10.1637/7821-122706-reg

Kuhn, J. H., Adkins, S., Agwanda, B. R., Al Kubrusli, R., Alkhovsky, S. V., Amarasinghe, G. K., Avšič-Županc, T., Ayllón, M. A., Bahl, J., Balkema-Buschmann, A., Ballinger, M. J., Basler, C. F., Bavari, S., Beer, M., Bejerman, N., Bennett, A. J., Bente, D. A., Bergeron, É., Bird, B. H., Blair, C. D., … Zhou, P. (2020). Taxonomic update for phylum *Negarnaviricota* (*Riboviria*: *Orthornavirae*), including the large orders *Bunyavirales* and *Mononegavirales*. *Archives of Virology*, *165*, 3023–3072. https://doi.org/10.1007/s00705-020-04731-2

Kwon, J.-S., Lee, H.-J., Jeong, S.-H., Park, J.-Y., Hong, Y.-H., Lee, Y.-J., & Song, C.-S. (2010). Isolation and characterization of avian metapneumovirus from chickens in Korea. *Journal of Veterinary Science*, *11*(1), 59–66. https://doi.org/10.4142/jvs.2010.11.1.59

Legnardi, M., Allée, C., Franzo, G., Cecchinato, M., & Brown, P. (2021). Research Note: Detection of Avian metapneumovirus subgroup C specific antibodies in a mallard flock in Italy. *Poultry Science*, *100*(7), 101186. https://doi.org/10.1016/j.psj.2021.101186

Lemaitre, E., Allée, C., Vabret, A., Eterradossi, N., & Brown P. A. (2018). Single reaction, real time RT-PCR detection of all known avian and human metapneumoviruses. *Journal of Virological Methods*, *251*, 61–68. https://doi.org/10.1016/j.jviromet.2017.10.010

Liman, M., & Rautenschlein, S. (2007). Induction of local and systemic immune reactions following infection of turkeys with avian Metapneumovirus (aMPV) subtypes A and B. *Veterinary Immunology and Immunopathology*, *115*(3-4), 273–285. https://doi.org/10.1016/j.vetimm.2006.12.001

Ling, R., & Pringle, C. R. (1988). Turkey rhinotracheitis virus: *In vivo* and *in vitro* polypeptide synthesis. *Journal of General Virology*, *69*(4), 917–923. https://doi.org/10.1099/0022-1317-69-4-917

Listorti, V., Lupini, C., Cecchinato, M., Pesente, P., Rossi, G., Giovanardi, D., Naylor, C. J., &

Catelli, E. (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 Pathology*, *43*(1), 51–56. https://doi.org/10.1080/03079457.2013.866212

Lupini, C., Cecchinato, M., Ricchizzi, E., Naylor, C. J., & Catelli, E. (2011). A turkey rhinotracheitis outbreak caused by the environmental spread of a vaccine-derived avian metapneumovirus. *Avian Pathology*, *40*(5), 525–530. https://doi.org/10.1080/03079457.2011.607428

Lwamba, H. C. M., Alvarez, R., Wise, M. G., Yu, Q., Halvorson, D., Njenga, M. K., & Seal, B. S. (2005). Comparison of full-length genome sequence of *Avian metapneumovirus* subtype C with other paramyxoviruses. *Virus Research*, *107*(1), 83–92. https://doi.org/10.1016/j.virusres.2004.07.002

Madbouly, H. M., Tamam, S. M., Hussein, A. S., Mady, W., & Arafa, A.-S. (2014). Immunomodular effect of fusion gene DNA vaccine of avian metapneumoviruses. *Journal of Applied Poultry Research*, *23*(3), 478–485. https://doi.org/10.3382/japr.2014-00963

Majó, N., Allan, G. M., O'Loan, C. J., Pagès, A., & Ramis, A. J. (1995). A sequential histopathologic and immunocytochemical study of chickens, turkey poults, and broiler breeders experimentally infected with turkey rhinotracheitis virus. *Avian Diseases*, *39*(4), 887–896. https://doi.org/10.2307/1592428

Marianna, A., Giovanni, F., Claudia, M. T., Zoi, P., Kostantinos, K., Mattia, C., & Ilias, C. (2019). Molecular epidemiology of infectious bronchitis virus and avian metapneumovirus in Greece. *Poultry Science*, *98*(11), 5374–5384. https://doi.org/10.3382/ps/pez360

Mase, M., Yamaguchi, S., Tsukamoto, K., Imada, T., Imai, K., & Nakamura, K. (2003). Presence of avian pneumovirus subtypes A and B in Japan. *Avian Diseases*, *47*(2), 481–484. https://doi.org/10.1637/0005-2086(2003)047[0481:poapsa]2.0.co;2

Mayahi, M., Momtaz, H., Jafari, R. A., & Zamani, P. (2017). Detection and subtyping avian metapneumovirus from turkeys in Iran. *Veterinary Research Forum*, *8*(2), 105–108.

McDougall, J. S., & Cook, J. K. (1986). Turkey rhinotracheitis: Preliminary investigations. *Veterinary Record*, *118*(8), 206–207. https://doi.org/10.1136/vr.118.8.206

Mernizi, A., Bouziane, S., Fathi, H., Criado, L.G., Bouslikhane, M., Ghram, A., Catelli, E., Mouahid, M., & Nassik, S. (2022). First seroepidemiological and risk factor survey of avian metapneumovirus circulation in Moroccan broiler farms. *Veterinarski Glasnik*, *77*(1), 51-68. https://doi.org/10.2298/VETGL220307009M

Mescolini, G., Lupini, C., Franzo, G., Quaglia, G., Legnardi, M., Cecchinato, M., Tucciarone, C. M., Blanco, A., Turblin, V., Biarnés, M., Tatone, F., Falchieri, M., & Catelli, E. (2021). What is new on molecular characteristics of Avian metapneumovirus strains circulating in Europe? *Transboundary and Emerging Diseases*, *68*(3), 1314–1322. https://doi.org/10.1111/tbed.13788

Mo, J., Angelichio, M., Gow, L., Leathers, V., & Jackwood, M. W. (2022). Quantitative real-time PCR assays for the concurrent diagnosis of infectious laryngotracheitis virus, Newcastle disease virus and avian metapneumovirus in poultry. *Journal of Veterinary Science*, *23*(2), e21. https://doi.org/10.4142/jvs.21153

Morley, A. J., & Thomson, D. K., (1984). Swollen-head syndrome in broiler chickens. *Avian Diseases*, *28*(1), 238–243.

Nagy, A., Abdallah, F., Sweed, K., Salama, A., & Omar, M. (2018). Seroprevelance of avian metapneumovirus in Egyptian chicken and duck flocks with a reference on economic impact. *Journal of Virological Sciences*, *4*, 8–14.

Naylor, C. J., & Jones, R. C. (1993). Turkey rhinotracheitis: A review. *Veterinary Bulletin*, *63*, 439–449.

Naylor, C. J., Shaw, K., Britton, P., & Cavanagh, D. (1997). Appearance of type B avian pneumovirus in Great Britain. *Avian Pathology*, *26*(2), 327–338. https://doi.org/10.1080/03079459708419215

Naylor, C. J., Worthington, K. J., & Jones, R. C. (1997). Failure of maternal antibodies to protect young turkey poults against challenge with turkey rhinotracheitis virus. *Avian Diseases*, *41*(4), 968–971. https://doi.org/10.2307/1592354

Ongor, H., Karahan, M., Kalin, R., Bulut, H., & Cetinkaya, B. (2010). Detection of avian metapneumovirus subtypes in turkeys using RT-PCR. *Veterinary Record*, *166*(12), 363–366. https://doi.org/10.1136/vr.c1607

Owoade, A. A., Ducatez, M. F., & Muller, C. P. (2006). Seroprevalence of avian influenza virus, infectious bronchitis virus, reovirus, avian pneumovirus, infectious laryngotracheitis virus, and avian leukosis virus in Nigerian poultry. *Avian Diseases*, *50*(2), 222–227. https://doi.org/10.1637/7412-071505r.1

Pedersen, J. C., Reynolds, D. L., & Ali, A. (2000). The sensitivity and specificity of a reverse transcriptase-polymerase chain reaction assay for the avian pneumovirus (Colorado strain). *Avian Diseases*, *44*(3), 681–685. https://doi.org/10.2307/1593111

Pringle, C. R. (1998). Virus taxonomy — San Diego 1998. *Archives of Virology*, *143*, 1449–1459. https://doi.org/10.1007/s007050050389

Qingzhong, Y., Barrett, T., Brown, T. D., Cook, J. K., Green, P., Skinner, M. A., & Cavanagh, D. (1994). Protection against turkey rhinotracheitis pneumovirus (TRTV) induced by a fowlpox virus recombinant expressing the TRTV fusion glycoprotein (F). *Vaccine*, *12*(6), 569–573. https://doi.org/10.1016/0264-410x(94)90319-0

Rautenschlein, S., Aung, Y. H., & Haase, C. (2011). Local and systemic immune responses following infection of broiler-type chickens with avian Metapneumovirus subtypes A and B. *Veterinary Immunology and Immunopathology*, *140*(1-2), 10–22. https://doi.org/10.1016/j.vetimm.2010.11.003

Retallack, H., Clubb, S., & DeRisi, J. L. (2019). Genome sequence of a divergent avian metapneumovirus from a monk parakeet (*Myiopsitta monachus*). *Microbiology Resource Announcements*, *8*(16), e00284-19. https://doi.org/10.1128/mra.00284-19

Rizotto, L. S., Simão, R. M., Scagion, G. P., Simasaki, A. A., Caserta, L. C., Benassi, J. C., Arns, C. W., & Ferreira, H. L. (2019). Detection of avian metapneumovirus subtype A from wild birds in the state of São Paulo. *Brazilian Journal of Veterinary Research*, *39*(3), 209–213. https://doi.org/10.1590/1678-5150-PVB-6021

Roussan, D. A., Haddad, R., & Khawaldeh, G. (2008). Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan. *Poultry Science*, *87*(3), 444–448. https://doi.org/10.3382/ps.2007-00415

Rubbenstroth, D., & Rautenschlein S. (2009). Investigations on the protective role of passively transferred antibodies against avian metapneumovirus infection in turkeys. *Avian Pathology*, *38*(6), 427–436. https://doi.org/10.1080/03079450903349204

Seal, B. S. (1998). Matrix protein gene nucleotide and predicted amino acid sequence demonstrate that the first US avian pneumovirus isolate is distinct from European strains. *Virus Research*, *58*(1-2), 45–52. https://doi.org/10.1016/s0168-1702(98)00098-7

Seifi, S., & Boroomand, Z. (2015). The role of avian metapneumovirus in respiratory complex disease circulating in broilers in Northern Iran. *Trakia Journal of Science*, *13*(2), 175–179. https://doi.org/10.15547/tjs.2015.02.011

Sheikhi, N., & Masoudian, A. (2011). Seroprevalence of avian metapneumovirus infection in some broiler breeder flocks of Iran. *Journal of Comparative Pathobiology Iran*, *8*(1), 431–437.

Shin, H.-J., Cameron, K. T., Jacobs, J. A., Turpin, E. A., Halvorson, D. A., Goyal, S. M., Nagaraja, K. V., Kumar, M. C., Lauer, D. C., Seal, B. S., & Njenga, M. K. (2002). Molecular epidemiology of subgroup C avian pneumoviruses isolated in the United States and comparison with subgroup A and B viruses. *Journal of Clinical Microbiology*, *40*, 1687–1693. https://doi.org/10.1128/jcm.40.5.1687-1693.2002

Shin, H.-J., Njenga, M. K., McComb, B., Halvorson, D. A., & Nagaraja. K. V. (2000). Avian pneumovirus (APV) RNA from wild and sentinel birds in the United States has genetic homology with RNA from APV isolates from domestic turkeys. *Journal of Clinical Microbiology*, *38*(11), 4282–4284. https://doi.org/10.1128/jcm.38.11.4282-4284.2000

Shin, H.-J., Rajashekara, G. F., Jirjis, F., Shaw, D. P., Goyal, S. M., Halvorson, D. A., & Nagaraja, K. V. (2000). Specific detection of avian pneumovirus (APV) US isolates by RT-PCR. *Archives of Virology*, *145*, 1239–1246. https://doi.org/10.1007/s007050070123

Sid, H., Benachour, K., & Rautenschlein, S. (2015). Co-infection with multiple respiratory pathogens contributes to increased mortality rates in Algerian poultry flocks. *Avian Diseases*, *59*(3), 440–446. https://doi.org/10.1637/11063-031615-case.1

Śmiałek, M., Kowalczyk, J., Gesek, M., Kaczorek-Łukowska, E., Dziewulska, D., Tykałowski, B., & Koncicki, A. (2021). The influence of maternally derived antibodies on protection against aMPV/A infection in TRT vaccinated turkeys. *Poultry Science*, *100*(5), 101086. https://doi.org/10.1016/j.psj.2021.101086

Śmiałek, M., Pestka, D., Tykałowski, B., Stenzel, T., & Koncicki, A. (2015). Development of vaccine-induced immunity against TRT in turkeys depends remarkably on the level of maternal antibodies and the age of birds on the day of vaccination. *BMC Veterinary Research*, *11*, 28. https://doi.org/10.1186/s12917-015-0345-5

Śmiałek, M., Tykałowski, B., Dziewulska, D., Kowalczyk, J., & Koncicki, A. (2020). IFNγ production profile in turkeys of different immunological status after TRT vaccination. *Journal of Veterinary Research*, *64*(2), 239–245. https://doi.org/10.2478%2Fjvetres-2020-0040

Śmiałek, M., Tykałowski, B., Pestka, D., Welenc, J., Stenzel, T., & Koncicki, A. (2016). Three-step Anti-aMPV IgA expression profile evaluation in turkeys of different immunological status after TRT vaccination. *Polish Journal of Veterinary Sciences*, *19*(3), 509–518. https://doi.org/10.1515/pjvs-2016-0064

Sugiyama, M., Koimaru, H., Shiba, M., Ono, E., Nagata, T., & Ito, T. (2006). Drop in egg production in chickens by experimental infection with avian metapneumovirus strain PLE8T1 derived from swollen head syndrome and the application to evaluate vaccine. *Journal of Veterinary Medical Science*, *68*(8), 783–787. https://doi.org/10.1292/jvms.68.783

Sun, J., Wei, Y., Rauf, A., Zhang, Y., Ma, Y., Zhang, X., & Li, J. (2014). Methyltransferase-defective avian metapneumovirus vaccines provide complete protection against challenge with the homologous Colorado strain and the heterologous Minnesota strain. *Journal of Virology*, *88*(21), 12348–12363. https://doi.org/10.1128/jvi.01095-14

Sun, S., Chen, F., Cao, S., Liu, J., Lei, W., Li, G., Song, Y., Lu, J., Liu, C., Qin, J., & Li, H. (2014). Isolation and characterization of a subtype C avian metapneumovirus circulating in Muscovy ducks in China. *Veterinary Research*, *45*, 74. https://doi.org/10.1186/s13567-014-0074-y

Tamam, S. M., Hussein, A. S., Arafa, A. M., & Madbouly, H. M. (2015). Preparation and evaluation of inactivated avian Metapneumovirus vaccine from recently isolated Egyptian strain. *Journal of Applied Poultry Research*, *24*(2), 168–176. https://doi.org/10.3382/japr/pfv019

Timms, L. M., Jahans, K. L., & Marshall, R. N. (1986). Evidence of immunosuppression in turkey poults affected by rhinotracheitis. *Veterinary Record*, *119*(4), 91–92. https://doi.org/10.1136/vr.119.4.91

Toquin, D., Eterradossi, N., & Guittet, M. (1996). Use of a related ELISA antigen for efficient TRT serological testing following live vaccination. *Veterinary Record*, *139*(3), 71–72. https://doi.org/10.1136/vr.139.3.71

Toquin, D., Guionie, O., Jestin, V., Zwingelstein, F., Allee, C., & Eterradossi, N. (2006). European and American subgroup C isolates of avian

metapneumovirus belong to different genetic lineages. *Virus Genes*, *32*, 97–103. https://doi.org/10.1007/s11262-005-5850-3

Townsend, E., Halvorson, D. A., Nagaraja, K. V., & Shaw, D. P. (2000). Susceptibility of an avian pneumovirus isolated from Minnesota turkeys to physical and chemical agents. *Avian Diseases*, *44*(2), 336–342. https://doi.org/10.2307/1592548

Tucciarone, C. M., Andreopoulou, M., Franzo, G., Prentza, Z., Chaligiannis, I., & Cecchinato, M. (2017). First identification and molecular characterization of *avian metapneumovirus* subtype B from chickens in Greece. *Avian Diseases*, *61*(3), 409–413. https://doi.org/10.1637/11631-032017-caser

Tucciarone, C. M., Franzo G., Lupini C., Alejo C. T., Listorti V., Mescolini G., Brandao P. E., Martini M., Catelli E., & Cecchinato M. (2018). *Avian Metapneumovirus* circulation in Italian broiler farms. *Poultry Science*, *97*(2), 503–509. https://doi.org/10.3382/ps/pex350

Tucciarone, C. M., Franzo, G., Legnardi, M., Pasotto, D., Lupini, C., Catelli, E., Quaglia, G., Graziosi, G., Dal Molin, E., Gobbo, F., & Cecchinato, M. (2022). Molecular survey on A, B, C and new avian metapneumovirus (aMPV) subtypes in wild birds of northern-central Italy. *Veterinary Science*, *9*(7), 373. https://doi.org/10.3390/vetsci9070373

Turpin, E. A., Stallknecht, D. E., Slemons, R. D., Zsak, L., & Swayne, D. E. (2008). Evidence of avian metapneumovirus subtype C infection of wild birds in Georgia, South Carolina, Arkansas and Ohio, USA. *Avian Pathology*, *37*(3), 343–351. https://doi.org/10.1080/03079450802068566

Umar, S., Teillaud, A., Aslam, H. B., Guerin, J. L., & Ducatez, M. F. (2019). Molecular epidemiology of respiratory viruses in commercial chicken flocks in Pakistan from 2014 through to 2016. *BMC Veterinary Research*, *15*, 351. https://doi.org/10.1186/s12917-019-2103-6

van Boheemen, S., Bestebroer, T. M., Verhagen, J. H., Osterhaus, A. D. M. E., Pas, S. D., Herfst, S., & Fouchier, R. A. M. (2012). A family-wide RT-PCR assay for detection of paramyxoviruses and application to a large-scale surveillance study. *PLOS One*, *7*(4), e34961. https://doi.org/10.1371/journal.pone.0034961

Van de Zande, S., Nauwynck, H., Cavanagh, D., & Pensaert, M. (1998). Infections and reinfections with avian pneumovirus subtype A and B on Belgian turkey farms and relation to respiratory problems. *Zentralblatt fur Veterinarmedizin Reihe B*, *5*(1-10), 621–626. https://doi.org/10.1111/j.1439-0450.1998.tb00835.x

Van de Zande, S., Nauwynck, H., Naylor, C., & Pensaert, M. (2000). Duration of cross-protection between subtypes A and B avian pneumovirus in turkeys. *Veterinary Record*, *147*(5), 132–134. https://doi.org/10.1136/vr.147.5.132

Velayudhan, B. T., Lopes, V. C., Noll, S. L., Halvorson, D. A., & Nagaraja, K. V. (2003). Avian pneumovirus and its survival in poultry litter. *Avian Diseases*, *47*(3), 764–768. https://doi.org/10.1637/7042

Velayudhan, B. T., McComb, B., Bennett, R. S., Lopes, V. C., Shaw, D., Halvorson, D. A., & Nagaraja, K. V. (2005). Emergence of a virulent type C avian metapneumovirus in turkeys in Minnesota. *Avian Diseases*, *49*(4), 520–526. https://doi.org/10.1637/7388-052805r.1

Villarreal, L.Y., Brandão, P. E., Chacón, J. L., Assayag, M. S., Maiorka, P. C., Rafi, P., Saidenberg, A. B., Jones, R. C., & Ferreira, A. J. (2007). Orchitis in roosters with reduced fertility associated with avian infectious bronchitis virus and avian metapneumovirus infections. *Avian Diseases*, *51*(4), 900–904. https://doi.org/10.1637/7815-121306-regr4.1

Wei, L., Zhu, S., Yan, X., Wang, J., Zhang, C., Liu, S., She, R., Hu, F., Quan, R., & Liu, J. (2013). Avian metapneumovirus subgroup C infection in chickens, China. *Emerging Infectious Diseases*, *19*(7), 1092–1094. https://doi.org/10.3201/eid1907.121126

World Organisation for Animal Health. (2022). *Turkey rhinotracheitis (avian metapneumovirus*

infections). WOAH. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.03.15_TURKEY_RHRHI.pdf

Youssef, N. M. A., & Ahmed, M. H. H. (1996). Serological studies on flocks showing depressed egg production. *Veterinary Medical Journal Giza*, *44*(4), 719–726.

Yu, M., Xing, L., Chang, F., Bao, Y., Wang, S., He, X., Wang, J., Wang, S., Liu, Y., Farooque, M., Pan, Q., Wang, Y., Gao, L., Qi, X., Hussain, A., Li, K., Liu, C., Zhang, Y., Cui, H., … Gao, Y. (2019). Genomic sequence and pathogenicity of the first avian metapneumovirus subtype B isolated from chicken in China. *Veterinary Microbiology*, *228*, 32–38. https://doi.org/10.1016/j.vetmic.2018.11.009

Yu, Q., Roth, J. P., Hu, H., Estevez, C. N., Zhao, W., & Zsak, L. (2013). Protection by recombinant Newcastle disease viruses (NDV) expressing the glycoprotein (G) of avian metapneumovirus (aMPV) subtype A or B against challenge with virulent NDV and aMPV. *World Journal of Vaccines*, *3*(4), 130–139. https://doi.org/10.4236/wjv.2013.34018

Zahirabadi, S. J., Akbariazad, G., Hosseini, H., Ghalavand, M., Tat, M., Hashemzadeh, M. S., & Dorostkar, R. (2017). Detection and molecular identification of avian metapneumovirus in commercial flocks of Qazvin. *Bioscience Biotechnology Research Communications*, *10*(2), 100-104.

Zhang, L., Peeples, M. E., Boucher, R. C., Collins, P. L., & Pickles, R. J. (2002). Respiratory syncytial virus infection of human airway epithelial cells is polarized, specific to ciliated cells, and without obvious cytopathology. *Journal of Virology*, *76*(11), 5654–5666. https://doi.org/10.1128/jvi.76.11.5654-5666.2002

Zhang, Y., Sun, J., Wei, Y., & Li, J. (2016). A reverse genetics approach for the design of methyltransferase-defective live attenuated avian metapneumovirus vaccines. In S. Thomas (Ed.), *Vaccine design: Methods in molecular biology* (Vol. 1404, pp. 103–121). Humana. https://doi.org/10.1007/978-1-4939-3389-1_7

Zuo, Z. H., Zhang, T. Y., Guo, Y. X., Chu, J., Qu, G. G., Miao, L. Z., & He, C. (2018). Serosurvey of *Avian metapneumovirus*, *Orithobacterium rhinotracheale*, and *Chlamydia psittaci* and their potential association with avian airsacculitis. *Biomedical and Environmental Sciences*, *31*(5), 403–406. https://doi.org/10.3967/bes2018.053