AVIAN METAPNEUMOVIRUS (AMPV)

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INTRODUCTION

Avian Metapneumovirus (aMPV) is associated with upper respiratory system infections of commercial birds, causing a respiratory disease mainly in turkeys and chickens. However, other species, such as pheasants and domestic ducks, can also be affected. The infection is characterized by mild to moderate respiratory symptoms, low mortality rate, and reproductive disorders in laying or breeding hens. aMPV is a primary agent that encourages secondary infections due to opportunistic bacteria. Thus, increasing the mortality rate in affected birds is recognized as one of the limiting factors of poultry production worldwide. The economic impact is associated with losses for delaying growth and/or decreasing egg production and quality. Thus, MPV is associated with Avian Respiratory Complex (ARC) and Swollen Head Syndrome (SHS) in chickens and is the agent causing the disease recognized worldwide as Turkey Rhinotracheitis (TRT)(1,2).

Since its detection in the 70s, aMPV has been reported in most regions worldwide, especially in chicken and turkey production areas. Six subtypes are recognized: aMPV-A, aMPV-B, aMPV-C, aMPV-D, GuMPV B25, and PAR-05, whose distribution is associated with specific regions (3). Subtypes A and B are prevalent in Europe, Africa, Asia, and some Latin American countries such as Brazil and Mexico, affecting chickens in different production phases. Subtype C is the most prevalent in turkey-producing states in the United States and Canada. Furthermore, it has been found in China, Korea, and France but with genomic differences, which divided it into lineages: American and Eurasian (Figure 1). The new GuMPV and PAR-05 subtypes were identified in wild birds in Canada and the United States, respectively (4).

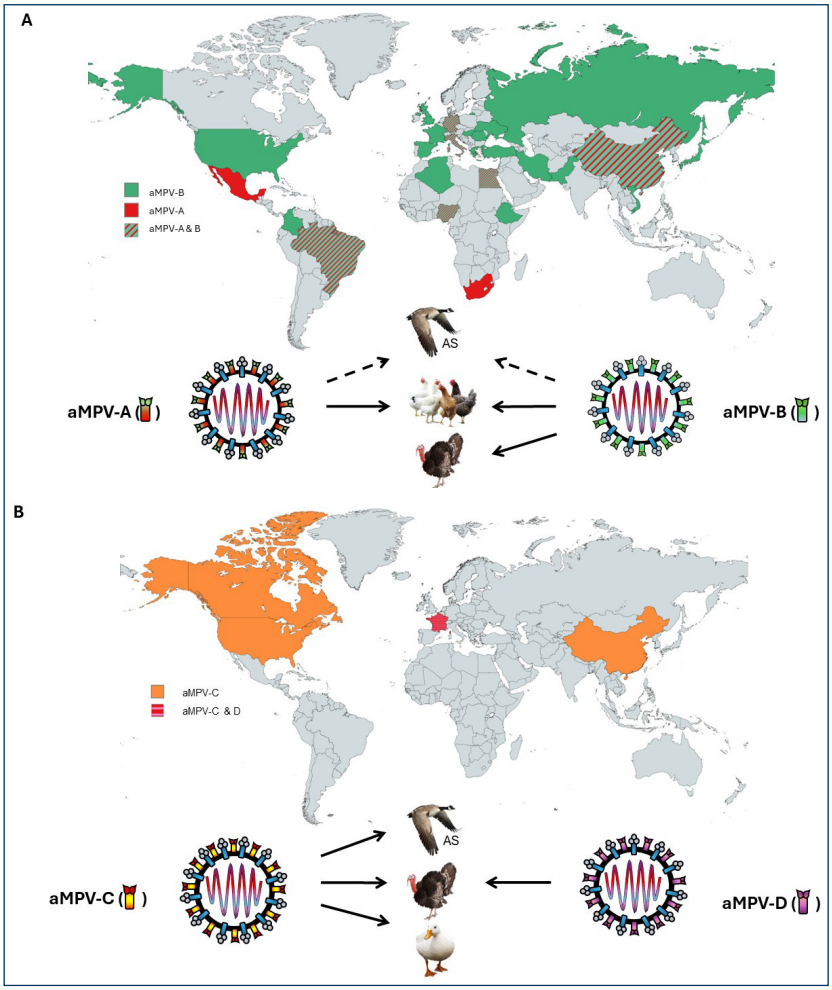

Figure 1. Geographical distribution and hosts of the six aMPV subtypes described around the world. The full arrows show that the subtype causes disease in indicated birds, and the segmented arrow shows that birds are virus carriers. AS: Wild birds. (a) American lineage of aMPV-C. (b) Eurasian lineage of aMPV-C.

ETIOLOGY

aMPV is an enveloped single-stranded RNA virus with a negative polarity that belongs to the Pneumoviridae family and the Metapneumovirus genus, in which two species are recognized: aMPV and human Metapneumovirus (hMPV). The aMPV genome has a variable size, ranging between 13 to 15 kb, depending on the subtype (5).

It encodes eight structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), M2 protein (M2), glycoprotein G (G), small hydrophobic protein (SH), and large polymerase protein (L), organized with the following arrangement: 3'-N-P-M-F-M2-SH-G-L-5' (Figure 2).

The N protein wraps and protects the viral RNA, forming the nucleocapsid, the most crucial component of the viral capsid. The P, M2, and L proteins are associated with nucleoprotein and form the ribonucleoprotein complex. The P protein acts as a cofactor of the L protein and, in aMPV-C, has been shown to play a role in inhibiting the host interferon (INF) response. This antagonistic interaction with the immune system can facilitate virus replication, increasing the viral load excreted into the environment and, subsequently, more significant damage to respiratory tissue. The M protein organizes the assembly of the virus and wraps the nucleoprotein. This protein is covered by a lipid layer that contains the three membrane glycoproteins: F, SH, and G. These three glycoproteins of the virus are considered determining factors of tropism, antigenicity, and virulence. After fusion, the viral nucleocapsid enters the host cytoplasm for replication, where the N, P, M, M2, and L proteins form the polymerase complex, responsible for most of the enzymatic processes involved in transcription and replication. Differences in nucleotide sequence, amino acids, and genome size have been identified between aMP subtypes. The glycoprotein G gene is recognized as the most variable, and the N gene is the best preserved (6).

F protein is a membrane glycoprotein that mediates infection by facilitating fusion between the viral envelope and host cell membranes. This protein is recognized as the primary determinant of virus tropism in the respiratory system cells of birds. This glycoprotein is also responsible for virus fusion with αvβ1 integrin receptors of the host's respiratory system cells to initiate the infection. Glycoprotein G, in turn, has been related to the evasion of the immune system and the adhesion of the virus to the target cell membrane. SH protein is another glycoprotein proposed to act as a viroporin, facilitating virus invasion into host cells by increasing the permeability of the cell membrane (7–9).

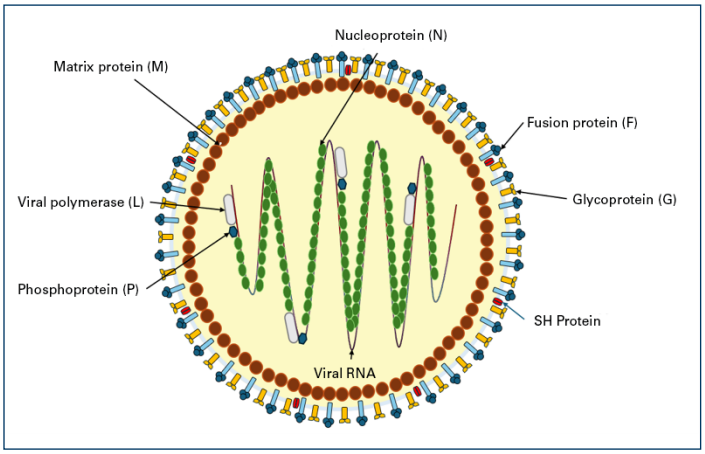

Figure 2. Viral structure representation of aMPV. The image shows the structural proteins encoded in the viral genome: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein G (G), small hydrophobic protein (SH), and large polymerase protein (L). The M2 protein (M2) is part of the eight structural proteins.

LESIONS, CLINICAL SIGNS, AND TRANSMISSION

aMPV infection begins with virus replication in the respiratory epithelium and the recruitment of lymphoid cells, causing lesions in the upper respiratory system mucosa. Damage to respiratory tissue is characterized by epithelial desquamation, loss of ciliary activity, and necrosis, which allows colonization by secondary bacteria (10,11).

In turkeys, the disease shows excessive nasal and ocular discharge, sneezing, rales, and inflammation of the infraorbital sinuses, facilitating the virus's excretion into the environment. In severely affected birds, dyspnea (open beak breathing) and sneezing are caused by nares blockage with mucoid content. Generally, symptoms shown in infected turkeys have been mainly associated with aMPV-C; however, it has been confirmed that aMPV-B infection in the field similarly manifests itself (12,13).

In chickens, aMPV infection diagnosis is more challenging because the disease shows less obvious respiratory signs than those commonly associated with aMPV-A and B subtypes. In some cases, eye and nasal secretions and inflammation of the periorbital tissue and infraorbital sinuses can be observed. Neurological signs such as torticollis, disorientation, and opisthotonos may occur in severe cases. Birds often will not develop apparent symptoms, and they go unnoticed. On the other hand, alterations such as discoloration and fragility of the shell can be observed in laying hens and turkeys, along with a decrease in egg production of 30% to 70%. These reproductive disorders can appear with or without respiratory symptoms (14,15).

It is essential to consider that respiratory and reproductive signs are not unique to aMPV, since other viral agents related to CRA, such as Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (AIB), and Avian Laryngotracheitis (ALT), as well as bacteria such as *Mycoplasma* spp, *Ornithobacterium rhinotracheale*, *Avibacterium paragallinarum*, among others, can produce similar clinical signs. Likewise, aMPV infection can be exacerbated by bacteria such as *Escherichia coli*, *Bordetella avium*, and *Mycoplasma gallisepticum*. It can also be found in infections with viruses such as infectious bursal disease virus (IBDV), IBV, and NDV (16).

The virus is transmitted horizontally, associated with failures in biosecurity, because of direct contact with the secretions and aerosols of infected animals or through contaminated fomites (bedding materials, food, water, vehicles, and people), which can also be involved in the transmission of the virus within the farm and its surroundings. In addition, wild birds have been confirmed to play a role in spreading the virus worldwide. This is due to the contact, in some cases close, of commercial birds with wild bird populations and/or contamination of nearby water bodies. To this date, there is not enough evidence to confirm vertical virus transmission (Figure 3).

Infected birds begin excreting the virus within three to five days after infection, and it can be extended for up to seven to nine days. Recovery of animals showing the disease without secondary infections can take up to two weeks, while egg quality and production disorders can take up to three weeks. The impact of infection on the virus depends on production age, and the laying phase is unknown (1).

DIAGNOSTIC STRATEGIES

Diagnosis begins with identifying respiratory or reproductive signs compatible with aMPV infection. Molecular detection is used more frequently than viral isolation, as it is a more sensitive and less expensive tool. However, virus detection is usually tricky due to the short excretion period in infected animals, which occurs between three to ten days post-infection (DPI). Observed symptoms can be mild to none. Based on the above, sampling for molecular diagnosis should be performed as soon as clinical signs are observed. Collecting respiratory and reproductive tract swabs, individually or in pools of no more than five individuals from the same barn, is recommended. In molecular tests, viral RNA can be detected at up to nine DPI levels in the trachea and fourteen DPI levels in nasal turbinates (17,18).

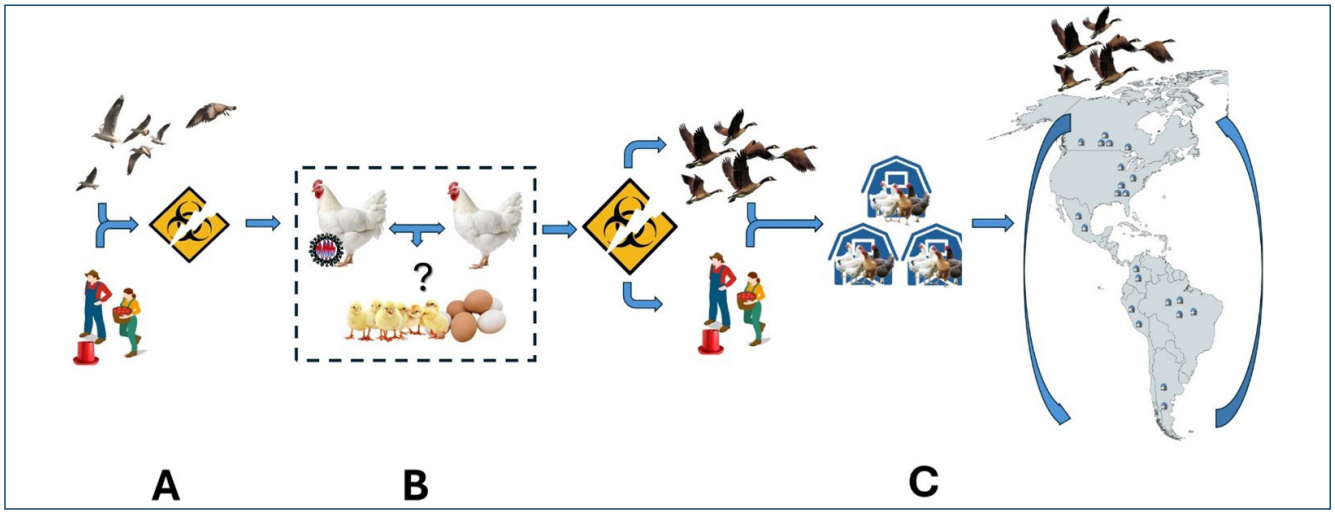

Figure 3: Proposed virus transmission epidemiological aspects. A: Entry scheme to a poultry production setting due to a possible biosecurity breach. B: Viral load increase caused by horizontal transmission between infected birds within a core, considering the unproven possibility of vertical transmission. C: Virus exit from one farm to neighboring farms and subsequently regional spread facilitated by migratory routes of wild birds.

Molecular detection of aMPV is done with reverse transcriptase PCR (RT-PCR). This test provides a sensitivity and specificity close to 100%, being ideal to identify virus subtypes. N and G genes have been used as amplification targets for aMPV detection and characterization. The N gene can provide a greater capacity for virus detection, even in wild birds; however, it does not allow the characterization of the detected subtypes. In turn, the G gene has been advantageous in detecting and differentiating subtypes A and B in countries where both subtypes may be circulating. Due to the preceding, detection strategies are proposed in two phases, where the N gene is used in the first instance, thus establishing the presence of any subtype, and after this, positive samples will be submitted to characterization, where the G gene is targeted. Characterization of the subtype present in the sample is almost as crucial as its detection since it allows us to propose and guide effective vaccine strategies for disease control in a region (19,20). Table 1 summarizes techniques available for aMPV diagnosis.

Depending on the type of productive system, a necropsy of respiratory organs (turbinate, trachea, lungs and air sacs) and reproductive organs can be performed to carry out histopathological diagnosis and viral isolation. In cases where a secondary infection occurs, giving rise to more extensive upper respiratory system tissue damage, the virus will likely no longer be detected. This is because while epithelium destruction caused by secondary infection progresses, the virus will gradually lose space to replicate. After all, there is a smaller proportion of relatively healthy cells. In this scenario, diagnosis can be complicated since clinical signs may be caused by the secondary infection, while it is less and less possible to detect the virus (1,21).

Serological diagnosis is beneficial in confirming infection in animals that have already gone through the viral excretion period, with indirect ELISA being the most widely used method. The sensitivity of the test will depend on the subtype and antigen used. It is necessary to consider the limitations in detecting antibodies and, therefore, in the serological diagnosis when antigens from heterologous strains of the present virus are used, even when there may be phylogenetic proximity between strains. Competitive ELISA kits have been developed using synthetic monoclonal antibodies that may increase the sensitivity and specificity of the test; however, it is essential for kit selection purposes to consider the circulating subtype in the region (22).

Table 1. Recommended strategies and tests for aMPV diagnosis

Diagnosis	Test	Sample	Remarks
Serological	Enzyme-linked immunoassay (ELISA)	Serum or whole blood to extract serum.	For results interpretation, it is important to consider the existence of maternal or vaccine antibodies. Commercial kits specific for A, B, or C subtypes are available.
Molecular	G gene RT-PCR	Swabs from: trachea, oropharynx, nares, and uterus. Tissues: nasal turbinates, trachea, lungs, and uterus.	**G and N Genes RT-PCR:** It is practical to detect active infections and characterize the subtype present in the sample **Sequencing:** makes possible differentiation of the origin of the strain present in the sample
	N gene RT-PCR		
	Whole genome sequencing (WGS)		
Virological	Viral isolation	Nasal turbinates, trachea, lungs, and uterus.	Requires preliminary confirmation of the presence of the virus by other techniques

CONTROL

One of the aMPV control strategies is based on vaccines, whose primary purpose is to reduce symptoms and mortality in turkeys and chickens against infection by virulent strains. Live attenuated and inactivated commercial vaccines have been developed and used in this connection. Live attenuated vaccines of subtypes A, B, and C are widely used in Europe, Asia, the United States, and some parts of Latin America. These vaccines induce a rapid local immune response followed by cellular immunity (3).

aMPV-B live attenuated vaccines can provide homologous and heterologous protection against aMPV-B and aMPV-A. Inactivated vaccines are also used as boosters for live vaccines, seeking to extend the protection of adult birds. It has been reported that maternal antibodies can delay or even interfere with the generation of vaccine antibodies, especially in chickens, where the humoral response tends to be poor after the first vaccination. However, this does not mean birds cannot develop immunity late after the vaccine is given (23).

Recently, innovative strategies have been proposed and evaluated for developing vaccines against aMPV, such as using other viruses to vector structural proteins of aMPV. Some examples include inserting the F protein gene into the genome of a fowl pox vaccine strain and the G protein gene into the genome of an NDV vaccine strain. However, in addition to vaccination, virus control must be accompanied by appropriate farm biosecurity measures (3).

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