Avian metapneumovirus (AMPV), also known as turkey rhinotracheitis virus, is one of the major respiratory pathogens in poultry, causing reduced productive performances and increased mortality, mainly due to secondary infections. This virus appeared for the first time in South Africa during the late 1970s and, since then, has spread worldwide. Turkeys and chickens of all ages are the natural hosts, but guinea fowls, pheasants and partridges are also sensitive. The main target tissue for viral replication is the upper respiratory tract; however, AMPV can also infect other organs, such as the oviduct. This results in typical respiratory clinical signs, but also in egg production drops in laying birds.

The disease can be exacerbated by other pathogens, such as *Escherichia coli, Mycoplasma, Chlamydophila* and, in chickens, AMPV and *E coli* co-infection can cause swollen head syndrome, which is characterised by an inflammation of the head subcutaneous tissue. Both clinical and postmortem findings are not specific as they can be similar to those caused by other viral respiratory pathogens. For this reason, viral identification is critical for a definitive diagnosis. Primary isolation of AMPV is possible, but not routinely practical, while reverse transcription PCR is commonly used to detect viral genome.

Furthermore, ELISA kits are commonly used to detect, serologically, the infection. As a therapy against AMPV is not available, a preventive approach is critical, both in avoiding the infection of birds and in controlling eventual losses caused by the disease, and vaccination is one of the main tools in controlling the disease. This article provides a general overview on the topic.

Avian metapneumovirus (AMPV) is one of the major respiratory pathogens in poultry, causing reduced performances and increased mortality, mainly due to secondary infections.
This virus first appeared in South Africa during the late 1970s and, since then, has spread worldwide. The disease was firstly identified in turkeys – leading to the pathogen being originally named turkey rhinotracheitis virus. However, as the virus can infect other avian hosts, avian rhinotracheitis is often used to indicate the disease in non-turkey species. According to the official nomenclature the virus belongs to the Paramyxoviridae family (including single-stranded, negative sense RNA and enveloped viruses), sub-family Pneumovirinae.

Previously known as avian pneumovirus, it finally became AMPV after molecular studies highlighted conspicuous differences compared to other pneumoviruses. Four subtypes of AMPV have been identified and named A, B, C and D. These have been classified using several techniques (for example, serological, sequencing and amino acidic analysis) and that is why we use the general word “subtype” and not more specific terms, such as serotype, genotype or pathotype.

AMPV has been detected worldwide, with the only exception of Oceania. Subtypes A and B are responsible for the disease in all affected continents, with the only exception being North America, where infections are caused only by AMPV subtype C. Subtype C-related strains have been more recently detected in France and China in Muscovy ducks and Korea in pheasants. Subtype D was isolated only once in France in 1985 and was identified at a later stage following retrospective analysis. It has never been detected again since.

**Hosts, pathogenesis and disease**

Turkeys and chickens of all ages are the natural hosts, with turkeys, in particular, seeming to be the most susceptible. Guinea fowls, pheasants and partridges are also sensitive to AMPV infections, while pigeons, geese and ducks were believed to be resistant. However, studies have suggested a sensitivity of waterfowl to subtype C. Antibodies against AMPV have also been detected in farmed ostriches in Africa. Wild birds’ sensitivity has been proved only for subtype C in the US, while for subtype A and B, this issue remains poorly understood.

Direct contact transmission seems to be the only demonstrated way of infection, both directly with infected animals or their respiratory discharges. Other transmission routes have been supposed – for example, vertical or through contaminated water, equipment, feed truck or personnel, but no real evidence was demonstrated until now. In the US, migratory species have been recognised as a possible viral source.
Figure 1. A young poult showing conjunctivitis after experimental infection with avian metapneumovirus. Image: Elena Catelli, University of Bologna.

The upper respiratory tract is considered to be not just the first replicative site of the virus, but also the main target tissue for viral replication. AMPV seems to have a particular tropism for the ciliated cells of the nasal cavities, conchas, infraorbital sinus and trachea. However, occasionally it can reach the lungs and air sacs.

Bacterial co-infections seem to facilitate viral penetration along the lower respiratory tract – Escherichia coli, Bordetella avium, Mycoplasma gallisepticum and imitans, Riemerella anatipestifer, Chlamydophila psittaci and Ornithobacterium rhinotraceale have all proved to exacerbate the disease and to enhance virus distribution in infected birds. It is still not clearly understood how the virus can infect other organs outside the respiratory system, but it is common to detect AMPV mainly in the reproductive tract and, on some occasions, in the Harderian gland, kidneys, spleen, cecal tonsil and bursa of Fabricius. A short, transient viraemia could explain this behaviour, although the virus has rarely been found in the circulation.

Some authors have suggested – supported by in vitro studies with subtype C – macrophage cells are particularly susceptible to AMPV and to possibly be responsible for viral dissemination. No clear differences have been found in the pathogenesis among different subtypes; different results seem more related to the virulence of the single strain than to the subtype belonging.

The clinical outcome of the infection is typically characterised by respiratory symptoms, such as coughing, sneezing, nasal discharge, swollen infraorbital sinus, but even conjunctivitis (Figure 1) and submandibular oedema can be present. Morbidity is usually very high, while mortality can be variable. The severity of the disease is highly dependent on management factors, including bird density, ventilation, temperature, hygienic conditions and on secondary bacterial infections.

Co-infections of AMPV and E coli have been associated in chickens with swollen head syndrome.
This is characterised not just by respiratory signs, but also by a general head swelling, which leads to neurological signs, such as disorientation, torticollis and opisthotonus. Infections cause a drop in egg production in laying birds, which are usually in the order of 10% to 20%, but can reach even 70% in turkeys. Egg quality is affected, showing poor, thin shells. Reduced performances have been reported in the field in laying hens, too – although, in experimental conditions, never after respiratory challenge. In contrast to the situation in turkeys, only intravenous injection of virus is able to decrease the laying performance in chickens. This difference between the two species has never been explained.

While in turkeys the gross lesions, due to uncomplicated infections, are a considerable finding, in chickens and other species, these are quite uncommon. In turkeys, lesions include the presence of watery to mucoid exudate in the upper respiratory tract, swelling of the infraorbital sinus caused by accumulation of mucus, conjunctivitis and submandibular oedema.

In breeders, prolapsed oviducts, folded shell membrane in the reproductive tract and egg peritonitis may be seen. Bacterial secondary infection can aggravate these findings, resulting in airsacculitis, pericarditis and perihepatitis. In chickens with swollen head syndrome, the head and neck may increase in size and be swollen due to an accumulation of a yellow gelatinous or even purulent oedema in the subcutaneous tissue. No major differences have been found microscopically in different species.

![Microscopic slides showing a normal tracheal mucosa (left), compared on the right to a mucosa infected with avian metapneumovirus, revealing loss of cilia and mononuclear infiltration. Image: Elena Catelli, University of Bologna.](image)

**Figure 2.** Microscopic slides showing a normal tracheal mucosa (left), compared on the right to a mucosa infected with avian metapneumovirus, revealing loss of cilia and mononuclear infiltration. Image: Elena Catelli, University of Bologna.

As stated previously, AMPV has a particular tropism for epithelial cells. The main histological lesions are located on the respiratory epithelium, characterised by deciliation, de-epithelisation, thickening of the mucosa, hyperaemia, mononuclear infiltration (**Figure 2**) and glandular proliferation in the turbinates, infraorbital sinuses and trachea. Lesions are usually transient and detectable in the first 10 days after infection. After three weeks, birds are totally recovered. Epithelial damages in the oviduct have been also observed.

The immune reactions of birds towards the infection have not been fully clarified. Some studies have speculated cellular-mediated immunity to be critical, for protection, while humoral immunity appears not to be critical, and circulating antibody titres do not seem to be an indicator of
protection. In experimental infections, turkeys with no detectable antibodies were protected against challenge with a virulent strain and vaccinated bursectomised poults were resistant to challenge.

Furthermore, suppression of T-lymphocytes with ciclosporin A caused delayed recovery from clinical signs and more lasting microscopic lesions. Local immunity could be related in resistance to infection; however, as suggested for other respiratory pathogens, its short duration might explain recurrent infections during birds’ productive lives in farms. Maternal antibodies are passed from hens to their progeny via the egg yolk, but their role does not seem to be significant, as they do not prevent infections nor interfere with early vaccination – allowing young chicks to be immunised in early stages.

**Diagnosis**

Both clinical and postmortem findings are not specific as they can be similar to those caused by other viral respiratory pathogens, such as Newcastle disease, infectious bronchitis, low pathogenic avian influenza, mycoplasmas or respiratory bacterial infections. Viral identification is, therefore, critical for a definitive diagnosis. This target can be reached directly, by isolating or detecting the virus or, indirectly, by demonstrating specific serological responses in the host.

AMPV has a very short persistence in the host before clearance. For this reason, virus isolation and detection are not always easy. Samples must be taken in the very early stages (at three to five days after infection) from birds not yet showing clinical signs.

Primary isolation of AMPV is possible using tracheal organ culture or embryonated eggs inoculated via the yolk sac. Both methods seem to have good sensitivity, but isolation of subtype C in tracheal organ culture is not suitable as this strain does not cause ciliostasis. Once isolated, AMPV can be easily adapted to grow in different cell lines, such as Vero or in chick embryo fibroblasts and chick embryo liver cell monolayers. The cytopathic effect is not specific and often characterised by the presence of small syncytia.
Viral detection is normally carried out by reverse transcription PCRs performed from oropharyngeal swabs (Figure 3), filter paper cards or tissues. Its high sensitivity enables viral presence to be revealed for a longer period compared to isolation methods. Several PCR protocols have been described, some of which are subtype-specific while others can detect all four subtypes. Real-time PCR protocols have also been developed – allowing not just a more sensitive viral detection, but even viral quantification.

As antibodies against AMPV have been proven to be detectable for at least 89 days in sera after infection, serological tests are commonly used to confirm infection, especially in commercial poultry.

ELISA is now the most common test due to its sensitivity, specificity and suitability for mass serological screening. Several commercial kits have been developed. Performances are highly dependent on the coated antigen; homologous tests have shown a higher efficiency compared to heterologous ones, especially among different subtypes. This can give rise to false negatives and the illusion a vaccine has not “taken”. Furthermore, subtype C antibodies are detected very poorly by specific subtype A and B ELISA.

**Control**
As a therapy against AMPV is not available, a preventive approach is critical both in avoiding the infection of birds and in controlling eventual losses caused by the disease. Attention to hygiene and biosecurity practices, ventilation, temperature, density, stress control, disinfection and good management procedures are all critical in reducing symptomatology and mortality. Antibiotics can be used to prevent secondary bacterial infection.

Infections can be prevented by vaccination. Several vaccines are available and commonly used in commercial birds. Live attenuated vaccines can be administrated by several methods (intranasal, eye drop, drinking water or spray) to all bird categories at early stages to prevent the respiratory symptomatology. While in broilers one administration seems to be fully protective, in growing turkeys, repeated vaccinations are required.

Laying birds are usually vaccinated prior to the onset of lay by injection of inactivated vaccines to avoid egg production losses. Good cross-protection has been reported between A and B subtypes. On the other hand, subtype C vaccines do not protect against A and B subgroups. Simultaneous vaccination with AMPV and other respiratory viruses (infectious bronchitis and Newcastle disease) is not advised by pharmaceutical companies, although experimental studies have shown no interference in protection onset.

Finally, live AMPV vaccines have proved to be able to revert to virulence after several passages in naïve populations and, for this reason, scientists are trying to develop new generation and recombinant vaccines, which, unfortunately, are not yet commercially available.

**Conclusion**

Despite almost 40 years having elapsed from the discovery of AMPV, many aspects of this pathogen are still unclear and its eradication still seems far off. Further studies will be needed to clarify the viral ecology, improve the detection of all four – and possibly unknown – subtypes, and the development of more effective vaccines.

**References**