

Avian pneumoviruses – an update

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Avian pneumoviruses (APV) are the cause of turkey rhinotracheitis (TRT), a highly contagious respiratory disease of turkeys, involving high morbidity and variable mortality with significant falls in egg production in breeder turkeys. They also cause respiratory infection in chickens and are reported to cause loss of egg production in breeders and layers.

Often the term avian rhinotracheitis (ART) is used for infection in both species. The precise role of APV as a primary pathogen in the chicken is less clear than in the turkey, since experimental infection of chicks with the virus usually produces very mild or asymptomatic disease.

However, APV infection has been associated with loss of egg production in layers and breeders and APV vaccines are effective in preventing such losses. Swollen head syndrome (SHS) is sometimes a consequence of infection of chickens with APV.

TRT was first described in South Africa in the late 1970s and soon appeared in Europe and the Middle East. APV infections in turkeys and chickens are now virtually worldwide in distribution and of considerable economic significance, particularly in

the turkey. Until 1997, there was no evidence of APV infection in North America.

However, a virus isolated from outbreaks of respiratory disease among turkeys in Colorado, which later appeared in and has persisted in Minnesota, was shown to be a pneumovirus that had some molecular differences compared with the strains seen elsewhere. This article reviews the current knowledge of the infections and provides updates on some recent developments in diagnosis and vaccines.

Causal virus

APV is a pleomorphic, enveloped, RNA virus with an outer envelope bearing fusion (F) and glycoprotein (G) spikes. Originally, it was thought that there was only one type of APV, but work in the early 1990s, using monoclonal antibodies and nucleotide sequencing of the G gene, showed that there were at least two subtypes, identified as A and B. Originally, type A was found in South Africa and the UK and type B in the rest of Europe. However, it is now recognised that both subtypes are present in the



Nasal discharge caused by avian pneumovirus infection.

UK, in continental Europe and in most other parts of the world where poultry are kept, except for the USA, Canada and Australia.

These viruses grow in tracheal organ cultures (TOC), where they cause ciliostasis; they also grow in fertile fowls' eggs inoculated via the yolk sac. Both subtypes have been isolated from and can infect chickens and turkeys.

Commercial vaccines have been produced to both types A and B and while, under experimental conditions, cross-protection offered by each vaccine against the other is generally good, the existence of two types does lead to difficulties in interpretation of ELISA tests, depending on the antigen used.

The American virus, persistent in Minnesota, has been shown by molecular studies that it differs from subtypes A and B, to a greater extent than A and B differ from each other. The US virus, called type C (Colorado), and was originally isolated in Vero cells or chick embryo fibroblasts, is not neutralised by antisera to types A or B, does not cause ciliostasis in TOC, and its antibodies are only detected by ELISAs with homologous antigen.

More recently, French viruses originally isolated in the mid-1980s from turkeys have been shown to be non-A, non-B and not type C and are called subtype D. Another APV isolated from Muscovy ducks in France is more closely related to C than the others.

Thus, it seems possible that more types of APV will be detected in due course. In 2000

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Electronmicrograph of avian pneumovirus showing irregularly shaped particles with a distinct surface fringe of spikes.



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a human metapneumovirus was first described and is now known to be worldwide. It is most closely related to the avian subtype C than A or B and is not known to affect poultry.

Most of the descriptions to follow refer to what is known about APV types A and B.

Hosts

APVs have been demonstrated in turkeys, chickens, pheasants, guinea fowl and ducks and antibodies have been detected in ostriches in Zimbabwe and herring gulls in Germany. It is suspected that wild birds may

be important in the transmission of APV over large distances. In the USA, several studies have detected APV subtype C infection in wild migratory birds. However, whether virus replicates in wild species and plays a part in transmission of these viruses over long distances is not yet known.

Spread

Following infection of chickens or turkeys, APV is shed from the respiratory tract, primarily the nares and trachea.

The virus can spread rapidly within a flock, suggesting the importance of droplet and aerosol transmission, although mechanical

means are likely to be significant between flocks. Following its introduction into a susceptible turkey population, APV infection spreads rapidly and frequently occurs as an explosive epizootic.

Although APV has been isolated from very young poults and experiments have shown that there are abundant amounts of virus in the oviduct epithelium of the infected turkey hens, there are no confirmed reports of egg transmission in either turkeys or chickens.

Little work has been done on the survival of APV strains away from the host, but they are easily killed by common disinfectants. American work which needs confirmation, suggests that subtype C APV may survive in litter for several weeks.

Other factors

Exacerbating factors for APV infection are the common ones for respiratory diseases and include high ammonia and dust levels in the atmosphere, overcrowding and intercurrent infections. Infectious agents that have been shown to have a synergistic effect include *Escherichia coli*, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Chlamydia*.

In contrast, when chicks are infected with both APV and infectious bronchitis virus (IBV), IBV limits the replication of APV but still allows a protective response and this has a bearing on vaccine programmes for young chicks. Newcastle disease vaccines do not appear to have the same effect. Dual infections with immunosuppressive viruses remain to be investigated.

E. coli infection is invariably present in swollen head syndrome (SHS) in broiler chickens, which may involve APV infection. However, APV is not the only virus associated with the condition and several reports indicate the involvement of IBV in some outbreaks. The mechanism has never been elucidated.

Development of disease

Susceptible turkeys and chickens become infected via the respiratory tract and virus replication can be detected by immunostaining of the epithelium of the turbinates and the trachea by two days postinfection. Infectious virus usually cannot be isolated from these sites for more than six to eight days and the lungs and air sacs do not usually contain virus.

However, intercurrent infections exacerbate and prolong the respiratory disease and permit greater penetration of virus into the lungs. In mature female turkeys without antibodies, virus has been demonstrated in the epithelium of the oviduct in all regions on days seven to nine postinfection. It is likely that virus reaches the oviduct via the bloodstream. Infection in laying chickens has been reported to cause loss of pigment in normally brown eggs.

Infection with APV, results in the development of virus neutralising and ELISA antibodies in the serum, but they do not appear to be important in controlling the respiratory disease but they do however, play an important role in protecting the oviduct after infection of laying birds and this is the rationale behind the use of killed vaccines.

Maternal antibodies have been shown to be ineffective in the face of early challenge of poults and chicks and they have little adverse influence on early vaccination with live vaccines. Local antibodies appear to be actively involved in protection and the tears have virus-neutralising activity.

Signs and lesions

● Turkeys.

APV infection results in an acute disease of the upper respiratory tract in turkeys from an early age. The condition most commonly affects turkey flocks aged between 3-10 weeks and is characterised by rapid onset, with high flock morbidity that frequently approaches 100%.

Clinical signs may include depression, change of voice, gasping, moist tracheal râles, snicking, coughing, submandibular oedema, swollen infraorbital sinuses, foamy ocular discharge and excess mucus detectable at the external nares.

In uncomplicated cases recovery is rapid and may be complete in 7-14 days with low or no mortality. However, high mortality rates, often exceeding 50%, have been reported and these are associated with secondary bacterial invasion, particularly by *E. coli*. Mortality rates may also be increased by poor hygiene or ventilation, over stocking and cold, damp weather.

In breeding flocks clinical signs may be less severe and are closely followed by a drop in egg production. Typically the loss is about 50%, but this may vary considerably. Egg production usually returns to normal in 2-4 weeks and the recovery phase is commonly associated with increased number of white and thin-shelled eggs. Reduction in hatchability may occur.

At necropsy, excess respiratory mucus is found in the nares and trachea which at first is clear but may become mucopurulent with time, especially where bacteria are involved. In complicated outbreaks typical lesions of colisepticaemia are found in several organs. In affected breeders, the oviducts may contain masses of inspissated albumen and occasionally solid yolk. Oviduct regression may be accompanied by egg peritonitis.

● Chickens.

The role of APV as a primary pathogen of the chicken is poorly understood. Strains of virus that cause overt clinical disease in turkeys induce an antibody response in chickens but only mild disease, with a clear nasal exudate, or subclinical infection. In broilers, APV may be one of several agents involved in respiratory disease of multiple

aetiology. Experimentally, concurrent infection of chicks with APV and other agents such as *E. coli* or *Mycoplasma gallisepticum* has been shown to exacerbate and prolong respiratory signs and lesions. In uncomplicated APV infection, microscopic lesions in the respiratory tract are usually less marked than in the turkey.

In breeders or commercial layers, APV infection is associated with reduced egg production and loss of shell colour. SHS consisting of swelling of the periorbital and infraorbital sinuses with associated torticollis and cerebral disorientation, usually in less than 4% of chickens in a flock, has been associated with earlier APV infection. SHS is not exclusively the result of APV infections and appears to be the consequence of secondary bacterial infection, usually *E. coli*.

Diagnosis

Clinical signs due to APV infection in the turkey and the chicken may resemble those caused by other respiratory agents. The diagnosis of APV infection depends on the demonstration of the virus or specific serum antibodies.

● Virus detection.

If virus isolation is attempted from clinical specimens, it is essential to obtain fresh material from affected birds in the early stages of the disease. Difficulty may be encountered in isolation since by the time clinical signs are evident, no infectious virus may be left.

Upper respiratory tract material is preferred to that from the trachea. Ocular, nasal or tracheal exudate or swabs bearing this material are agitated with antibiotic broth and inoculated into TOC. TOC are examined for up to 11 days for evidence of ciliostasis. A further one or two passages may be necessary if the samples are negative on first passage.

Virus identity may be confirmed by immunofluorescence staining of frozen sections or smears of infected TOC or by an SN test in TOC using an anti-APV serum. Molecular methods have been developed for detecting APV in clinical material.

A reverse transcriptase-polymerase chain reaction (RT-PCR) with appropriate primers is capable of distinguishing between types A, B and C. RT-PCR methodology has the advantages of high specificity and speed and is now widely used in diagnostic laboratories.

● Antibody detection.

ELISA is the method of choice for large-scale flock testing. Several commercial ELISAs are available for subtypes A and B viruses but discrepancies have arisen due to false-negatives, since ELISAs using plates coated with one antigen subtype may be less sensitive for detecting antibodies to the other subtype.

When birds have been vaccinated with

one type, and challenged with the other or perhaps both, interpretation of tests may be difficult. Furthermore, antibodies to the USA type C virus can only be detected with an ELISA using the homologous antigen.

In order to address the problems of ELISA sensitivity and specificity, newer approaches are being devised using molecular methods to express specific viral proteins important in ELISAs in *E. coli* or in insect viruses. This methodology will enable ELISAs to be tailored to detect antibodies specifically to individual subtypes if needed, or to all subtypes.

Control

There is no treatment for APV infection. The main approach to control has been through the use of vaccines. Live attenuated and inactivated oil adjuvanted vaccines generally give good protection, provided that they are given accurately, so that each bird receives the appropriate dose.

The live vaccines are used to protect against disease in growing turkeys and broilers and are effective in the face of maternal antibodies. They also act as primers for later application of killed vaccines.

Turkeys are usually vaccinated at day-old by spray, but broiler vaccination may be delayed by about one week, since there is evidence that simultaneous vaccination with live IB vaccine at day old impairs the response to APV vaccines. NDV vaccines do not present the same problems.

Sometimes turkeys are revaccinated during rearing by spray or in the drinking water, although the latter method is less effective. Although it has been shown that live vaccines developed from subtypes A and B protect against challenge with heterologous virus, vaccines of both types are frequently used for the same flock, and given alternately or simultaneously with half or full doses of each.

Sometimes adverse reactions have been reported following live vaccination. This may be due to incomplete vaccine cover, so that where a significant proportion of the flock remains unvaccinated, the virus may circulate and become more virulent.

To address this, several approaches to new vaccines are being developed, using the molecular techniques that are now available to us.

DNA vaccines and vector vaccines (poxvirus carrying selected APV genes) have been examined but perhaps the most promising is the use of reverse genetics to produce an infectious clone vaccine. Here a DNA copy is made of the RNA virus and the genes which are responsible for disease or immunity can be removed or modified.

Such viruses should not be capable of relatively quick reversion that the current vaccines may be capable of.

It can be expected that before long, vaccines based on these techniques will be nearing clinical trials. ■