CEVA HANDBOOK
OF POULTRY DISEASES

10 AVIAN METAPNEUMOVIRUS INFECTIONS

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DEFINITION

Infections with agents, etiologically Associated with avian meta-pneumovirus (aMPV) are turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in chickens. Clinically and morphologically, the conditions are characterised by serofibrinous inflammations affecting the upper respiratory tract, often complicated by the involvement of secondary pathogens.

HISTORY

The aMPV infection was established for the first time in 1978 as an expression of acute sinusitis in turkey poults aged 3–4 weeks in South Africa (Buys & du Preez, 1980). The symptoms of the condition included watery nasal discharges, which would then turn thicker and lead to congestion and swelling of the infraorbital sinuses. The disease was later discovered in a number of European countries: UK (Anon, 1985; Naylor et al., 1997), Spain (Anon, 1985), France (Giraud et al., 1986), Italy, Israel, Netherlands (Weisman et al., 1988), as well as other countries around the world: Mexico, Morocco, Taiwan, Brazil (Tanaka et al., 1995; El-Houadfi et al., 1991, Lu et al., 1994; Arns & Hafez, 1995). When aMPV first occurred in Europe and the Middle East, symptoms affecting the upper respiratory tract and a characteristic swelling of the head were observed in chickens. The condition was related to the identification of a pneumovirus, which by that time was designated as a turkey rhinotracheitis virus (TRTV) and the illness itself was named swollen head syndrome (Gough & Jones, 2008). In the early 1990s vaccinology based on turkey isolates began to develop. Two subtypes of aMPV (A and B) were proven to exist, with subtype A possibly providing protection against subtype B viruses (Cook, 2000). In the late 1990's aMPV was detected for the first time in the USA in turkeys, and was shown to belong to a subtype different from the already known A and B (Seal, 1998). The strains isolated later in America were found to be antigenically similar and were designated as aMPV subtype C. So far in North America, the aMPV infection has not been found in chickens and its spread among turkeys has been limited to several states (Bennet et al., 2004).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

Initially, the pathological agent was assigned to the Pneumovirus genus (Collinsetal, 1986). The virus exhibited some of the characteristics of a pneumovirus, yet it differed from mammalian pneumoviruses on a molecular level, and was therefore classified as a member of the Paramyxoviridae family, Pneumovirinae subfamily and Metapneumovirus genus (Pringle, 1998). The etiological agent is an enveloped RNA virus. The aMPV genome consists of approximately 13 kb single-stranded, non-segmented, negative-sense RNA, transcribing 8 genes responsible for the synthesis of structural and non-structural proteins (Banet-Noach et al., 2005). The target cells for replication are the ciliary epithelial cells of the respiratory tract (primarily the conchae and the trachea, as well as air sacs and lungs) (Jones et al., 1988; Majo et al., 1995). Viral replication occurs in the cytoplasm, catalysed by RNA/RNA polymerase. The formed virions leave the cell through the plasmatic membrane and produce a cytopathic effect (syncytia).

The virions present a medium to low resistance. Early reports on aMPV isolates described them as sensitive to lipid solvents and susceptible to inactivation when treated at 56°C for 30 minutes (Zarkov, 2003). Studies on aMPV subtype C, however, provided evidence for survival at 4°C for up to 12 weeks, at 20°C for up to 4 weeks, and at 37°C for up to 2 days. Disinfectants, such as ethanol, phenol derivatives, quaternary ammonium salts, iodophores, sodium hydroxide, etc. are able to inactivate the virus (Gough & Jones, 2008).

Based on antigenic characteristics and molecular analysis, the various isolates were divided into 4 groups: A, B, C, and D (Cook & Cavanagh, 2002). A cross reaction between the subtypes has been proven with the ELISA test, employing monoclonal antibodies. Based on the same principle, significant antigenic differences between the strains of the different isolates were detected (Collins et al., 1993; Cook et al., 1993). The viruses of the A and B subgroups belong to the same serogroup and can infect both chickens and turkeys (Cook, 2000; Juhasz & Easton, 1994). Phylogenetic analyses between the four subtypes established that the viruses of subtypes A, B and D were

more closely related than those from subgroup C (Gough & Jones, 2008). The isolates from the USA belonged to subgroup C, whereas the isolates from Europe and the rest of the world belonged to the other three subgroups (Guionie et al., 2007).

EPIDEMIOLOGY

aMPV outbreaks have been reported in poultry breeding regions throughout the world, except Australia (Gough & Jones, 2008). Most susceptible, and utmost significant, are turkeys and chickens, even though the infection may also be encountered in ducks, pheasants and guinea fowl. Antibodies against aMPV have been detected in ostriches and seagulls as well, yet the virus has not been isolated in these species (Marien, 2007). Reports of the infection's spread are often based only on a serological examination (Cook, 2000; Cook & Cavanagh, 2002). This is a result of the difficulties involved in detecting and identifying the virus. In 1999 the isolation of a pneumovirus was reported in 42-week-old Muscovy ducks with respiratory signs and a drop in egg production (Toquin et al., 1999). The discovered isolate was confirmed as aMPV through reverse-transcriptase polymerase chain reaction (RT-PCR). RNA from aMPV was also isolated from the nasal conchae of mallard ducks and wild geese in the USA. Viral RNA was isolated in Canada geese (Brantacanadaensis), although no viruses were isolated from these birds (Woolcoock, 2008). It was suggested that the birds could play the role of symptomless carriers of aMPV, and thus represent a potential source of infection for domestic turkeys (Shin et al., 2001). Transmission occurs horizontally via direct contact. So far, the spread by contact from infected to susceptible birds has been confirmed only experimentally (Alexander et al., 1986). The fact that North America has remained disease-free for many years, whereas in Central and South America, Europe, and other locations aMPV has been endemic, gives reason to assume that transmission by contact is essential to the spread of aMPV (Gough & Jones, 2008). Equipment

and facilities may also play a role in this process (Marien 2007). There is no published evidence of vertical transmission, although the virus has been detected in the reproductive tract of egg-laying birds (Jones et al., 1988). aMPV may induce immunosuppression too (Liman, 2008).

In countries where the aMPV infection occurs for the first time, the spread is fast within the farms where it emerges, then spreading rapidly to other farms. A high density of birds in barns is considered to favour the fast spread of the infection (Jones, 1996). Migrating wild birds are also presumed to be a factor in transmission (Gough & Jones, 2008).

Predisposing factors include inadequate ventilation, higher air humidity within the buildings, overpopulation and other issues related to a bad microclimate.

CLINICAL SIGNS AND PATHOLOGY

Turkey rhinotracheitis

The first symptoms appear rapidly, and the infection may affect 100% of the flock within 24 hours. The mortality rate varies between 2% and 50%, and is higher among younger birds. Appearance of a secondary infection and poor management may see even greater mortality.

Turkeys of all ages are at risk, yet younger turkey poults are apparently more vulnerable and exhibit the disease with more severe signs and a higher mortality rate (Alexander et al., 1986). For egg-laying birds, a drop in egg production of up to 70% may be observed, as well as the production of eggs with defective shells (Jones et al., 1988). A seasonal pattern in prevalence has been observed, with peaks during spring and summer.

Fig.1

Serous catarrhal conjunctivitis and infraorbital oedema are potential first symptoms of TRT in young turkeys.

Fig.2

Inflammatory oedema may affect the entire periorbital area of the head, especially with the involvement of secondary pathogens (E. coli, M. gallisepticum, etc.) and may be accompanied by severe seromucinous nasal discharge and foamy conjunctivitis.

Swollen head syndrome in chickens

Swollen head syndrome (SHS) is a complicated infection in broilers and broiler breeders, where the primary aetiological agent is an aMPV, and the secondary agent is usually *E. coli*; the disease is characterized by respiratory and nervous signs.

SHS IN BROILER CHICKENS

Fig.3

In broiler chickens, SHS is generally observed after the 4th week of life. The first clinical signs are sneezing, cough, rales and conjunctivites. A profuse tear secretion, reddened conjunctivas and a characteristic oblong almond-like shape of eyes are observed.

Fig.4

The inflammatory exudate is initially transparent, but afterwards becomes topaque.

Fig.5

Subcutaneous oedemas in the head region, involving unilaterally the periorbital.

Fig.6

Bilaterally oedema affecting and the mandibular space.

Fig.7

After removal of the covering skin, deposits of serofibrinous exudate are observed.

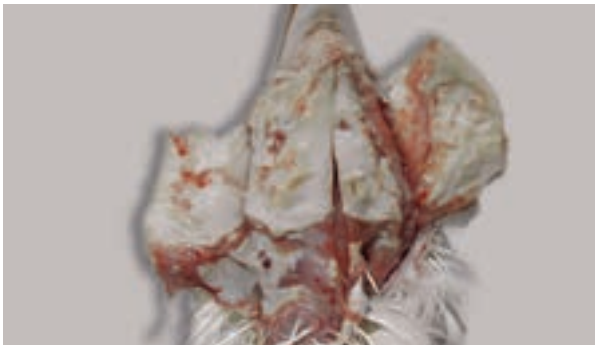

Fig.8

Massive deposits of fibrinous caseous exudate in the submandibular space. E. coli follows the APV infection of the upper respiratory tract. The resulting inflammatory response leads to accumulation of exudate in the subcutaneous tissue. In many instances, a croupous pneumonia develops at a later stage consequently to contamination with other pathogens.

SHS in broiler breeders

SHS in broiler breeders is usually encountered around or after the peak egg laying period, and only in female birds.

Fig.9

Unilateral swelling of the head is frequently observed, affecting the periorbital sinuses.

Fig.10

Bilateral swellings of the head, affecting the mandibular space and the wattles may be also observed.

Fig.11

Frequently, nervous signs are observed in broiler breeders (opisthotonus, torticolis) due to inflammatory processes in pneumatic skull bones and the middle ear.

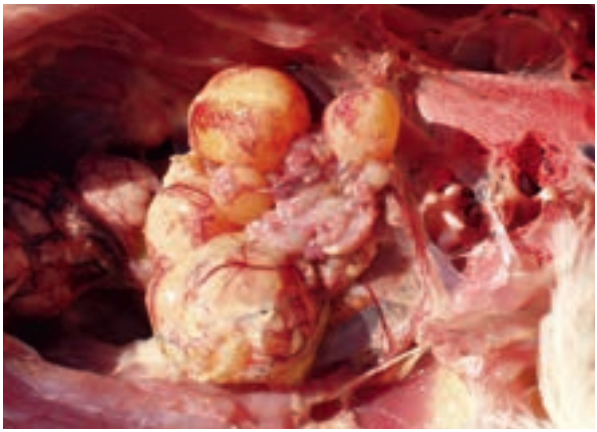

Fig.12

In laying hens, the ovaries are affected in many instances (serofibrinous oophoritis), resulting in reduced egg production.

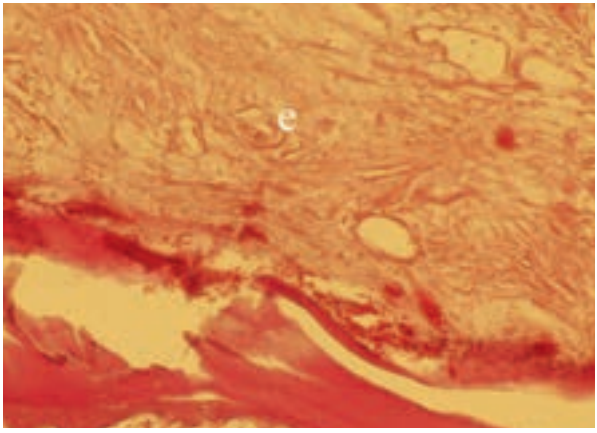

Fig.13

Subcutaneous mucinous oedema (e) in the region of the head in a broiler chicken. H/E, Bar = 70 µm.

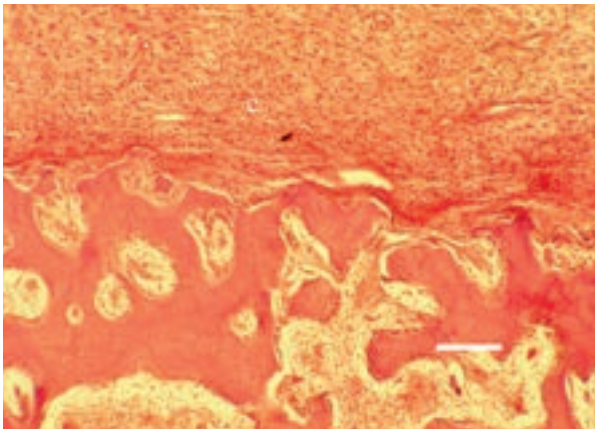

Fig.14

Subcutaneous inflammatory cell oedema (ic) in the region of the head in a female broiler breeder, 33 weeks of age, H/E, Bar = 35 µm.

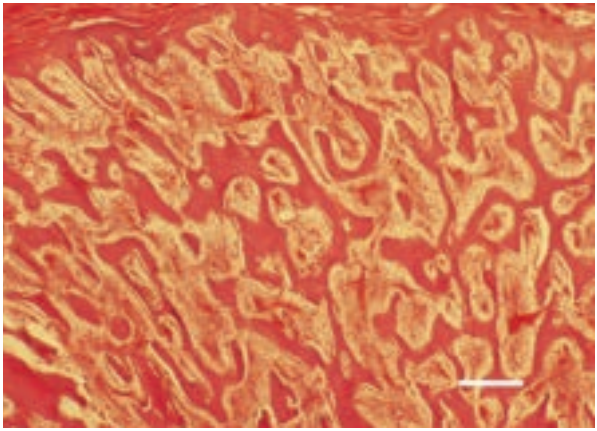

Fig.15

Haemorrhages and inflammatory necrotic lesions affecting the pneumatised skull bones in a female broiler breeder, 33 weeks of age, H/E, Bar = 70 µm.

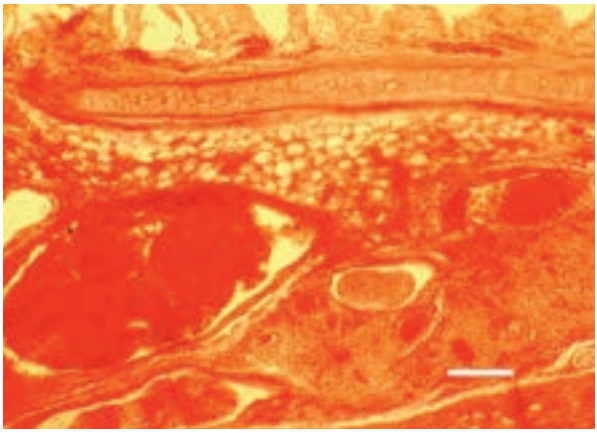

Fig.16

Massive haemorrhages and inflammation of nasal conchae in a 30-day-old broiler chicken. H/E, Bar = 100 µm.

DIAGNOSIS

The symptoms can indicate an aMPV infection, yet laboratory confirmation is always necessary. Diagnostic techniques (routine and molecular), as well as serological tests are used to identify the agent. The virus's fastidious nature requires that samples are collected as quickly as possible after the appearance of the first symptoms (Cook & Cavanagh, 2002). This is especially important when the subtypes have to be identified. The American subtype C is not associated with ciliostasis in tracheal organ cultures and is only cultured after numerous embryo and cell culture passages. Isolating a virus from birds with severe clinical symptoms is fairly rare. For other reasons which remain unclear, isolating the virus is much harder from chickens than from turkeys (Gough & Jones, 2008).

Samples for examinations should ideally be taken during the acute phase of infection, from the upper respiratory tract of live birds. Nasal secretions are also suitable subjects for examination, as are swab samples from the choanae, the conchae and the sinuses. The virus may also be isolated from the trachea and the lungs (Buys et al., 1989; Cook et al., 1999). The samples should be put into ice immediately after the collection, and if the time until the analysis is more than 72 hours, they must be kept frozen at -70°C.

Routine identification of the agent can be achieved by isolation of the virus from infected birds in tracheal organ cultures, or in turkey or chicken embryos followed by cultivation in a cell culture medium (Buys et al., 1989; Cook et al., 1999). Tracheal organ cultures can be prepared from turkey embryos originating from herds free of aMPV antibodies. Isolation through embryonated eggs is slow and requires repeated sampling. Embryonated 6–8 day old turkey or chicken eggs, inoculated in the yolk sac with material from infected birds, are used, followed by repeated analysis.

The molecular identification of aMPV is achieved using RT-PCR techniques. They are significantly faster and more sensitive than the standard methods for virus isolation. In order to detect endemic strains, subtypespecific RT-PCR procedures are used. Serological methods are the most commonly used to confirm aMPV infections, ELISA tests in particular. A number of commercial

ELISA kits are available for the examination of turkey and chicken sera. Competitive or blocking ELISA kits using monoclonal antibodies have also been developed. These kits are suitable for examinations of all aMPV subtypes. Apart from ELISA, virus neutralisation, micro-immunofluorescence and immuno-diffusion serological tests are also used.

DIFFERENTIAL DIAGNOSIS

aMPV infections must be differentiated clinically and morphologically from other viral or bacterial respiratory infections. Among other paramyxoviroses, we must consider Newcastle disease. The respiratory clinical signs, pathology and drop in egg laying are observed in infectious bronchitis and influenza as well. Another important illness with which aMPV must not be confused is the *M. gallisepticum* infection. The latter also tends to occur as a secondary complication of aMPV infections (Gough & Jones, 2008).

PREVENTION AND CONTROL

In order to prevent outbreaks of the disease, necessary preventive measures include specific and non-specific disease control procedures. The specific measures comprise vaccination of the flocks, and the non-specific include general management control.

Biosecurity

Separate rearing of the different age groups should be standard. The control of aMPV infections in poultry farms with mixed-age flocks is very difficult.

Of particular importance for the prevention and control of aMPV infections is compliance with the technological requirements for optimal microclimate in the buildings, provision of adequate ventilation and temperature, reasonable population density and suitable litter. General hygiene measures play an important role in reducing the spread of secondary pathogens.

Vaccination

Several factors should be taken into account when developing a vaccination programme, in order to achieve a long-lasting protective effect. Firstly, one cannot rely on immunity acquired from the maternal antibodies against aMPV infection (Cook et al., 1989; Naylor et al., 1997). This is why the vaccination programme should include vaccination as early as possible after hatching. Second, it is highly important to achieve a homogenous immunisation status for the whole flock, and indeed farm. It is important to ensure the application of the adequate dose of vaccine for the flock and the farm (Liman, 2008).

Two types of commercial vaccines are available to control aMPV infections: live attenuated and inactivated oil-emulsion vaccines. Currently, recombinant vaccines based on a vector gene of the fowlpox virus, expressing aMPV immunogenic protein F are under development (Stuart, 1989); as well as DNA vaccines through the usage of various aMPV proteins (Tanaka et al. 1995) and genetically attenuated aMPV produced via reverse genetics (Toquin et al., 1999).

The production of live vaccines involves attenuation of viral strains via repeated passages in embryonated eggs, tracheal organ cultures or cell cultures, or by combination of these methods. Live attenuated vaccines for commercial usage were produced from subgroups A and B of the aMPV isolates in Europe and subgroup C in the USA.

Live attenuated vaccines are used for immunisation of growing turkeys and chickens in order to induce an active immune response, which should protect them from aMPV respiratory conditions. They are also used as priming vaccines during the growth period in layer and breeder flocks, before the application of inactivated vaccines. It has been shown that live vaccines induce both local respiratory and systemic humoral immunity (Kehra, 1998). Live attenuated

vaccines are thought to be more powerful in inducing protective immunity, compared to inactivated ones. There are, however, several critical points to be taken into consideration beforehand: it has been confirmed that the protective immunity induced by live vaccines alone is very short, necessitating re-vaccination; field infections are often encountered despite the vaccinations; live vaccines may have residual immunosuppressive properties, which would facilitate certain bacterial or viral infections; there is a risk of emergence of more virulent variants of the strain used for attenuation (Liman, 2008).

Inactivated aMPV vaccines are used for booster immunisation after priming with live vaccines, with the purpose of acquiring high and homogenous levels of antibodies for a considerable period of time in layers and breeders. The strongest and most long-lasting protection is achieved through a combined priming – booster vaccination programme. This includes priming with live attenuated vaccines and booster immunisation with inactivated oil-adjuvant vaccines (Cook et al., 1995). Live vaccines are usually applied several times through coarse spray, in drinking water or by the nasal/ocular route. The first application is due no later than 7 days after hatching, and the second and third applications – at 3 and 6 weeks of age, respectively.

Inactivated aMPV vaccines are applied 4–6 weeks after the last live vaccine. Usually, this is 4 weeks before egg laying begins, which is at the age of 18 weeks for broiler breeders and 28 weeks for turkeys. Application is by individual intra-muscular injection in the breast or thigh muscle.

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