CEVA HANDBOOK
OF POULTRY DISEASES

7 MYCOPLASMA SYNOVIAE INFECTIONS

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DEFINITION

Mycoplasma Synoviae (MS) can cause respiratory disease, synovitis (inflammation in the lining of the joints), peritonitis, egg apical abnormalities (EAA) and subclinical infections.

HISTORY

Mycoplasma spp. are well-known pathogens in domestic birds causing significant economic losses (Lierz et al., 2007). A pathological condition related to *Mycoplasma* and determined as infectious synovitis in poultry was first described in the mid-20th Century (Olson et al., 1954). MS was sometimes reported as the cause of respiratory illness in chickens and turkeys, especially when the *MS* infection was combined with Newcastle Disease (ND), IB infections or vaccination (Jordan, 1979; Kleven & Ferguson- Noel, 2008). *MS* infection was also associated with a systemic infection in broiler chickens characterised mainly by sternal bursitis and aerosacculitis, the consequences of which are significant condemnation rates at slaughter (Senties-Cue et al., 2005). During the last decade a number of reports of eggshell apex abnormalities (EAA), also known as "glassy top eggs" related to *MS* have been published. EAA was first identified in the beginning of this century in commercial egg-layers from the Netherlands, and further reports emerged thereafter (Feberwee et al., 2009a; Catania et al., 2010). There had been no previous reports of EAA. Furthermore, the condition was reproduced through consequent inoculations of SPF chickens with IBV and *MS* strains isolated from the reproductive tract of birds exhibiting field EAA lesions (Feberwee et al., 2009a; Feberwee & Landman, 2010). An acceptable level of protection has been achieved using a live *MS* vaccine (Feberwee et al., 2009b).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

Mycoplasma synoviae is a Gram-negative microorganism and the pathology it causes leads to considerable economic losses for poultry operations. The primary sites of *MS* colonisation are the mucous

membranes of the respiratory tract and the joints of infected birds, although, as mentioned above, economic losses are increased by problems related to eggshell formation. The infection occurs mainly through bacteria attaching themselves to the host cells through specialised surface proteins and adhesion, resulting in intracellular persistence that may lead to death (Stippkovits, L. & I. Kempf, 1996).

MS is often the cause of subclinical infection of the upper respiratory tract, which may progress into a respiratory illness with lesions in the air sacs, especially in cases of coinfection with other pathogens, such as NDV, IBV etc., or when a greater number of virulent *MS* strains are involved.

MS requires nicotinamide adenine dinucleotide (NAD), and the NAD-reducing agent cysteine hydrochloride in addition to the complex of nutritional requirements of mycoplasmae.

MS has two main surface antigens, MSPA and MSPB, and it has been proven that MSPA is a haemagglutinin and MSPB – a lipoprotein (Noormohammadi et al., 1997). These antigens are coded through a single *vlhA* gene (variable lipoprotein and haemagglutinin), probably with post-translation splitting to separate the two proteins. The coded sequence of the *vlhA* gene is homologous to the sequence for the *vlhA* genes of MG, even though *MS* is philogenetically different from MG (Noormohammadi et al., 2002). Similarly to *VlhA* (previously called pMGA) of MG, the haemagglutinin MSPA is an object of high-frequency phase and antigen variation (Noormohammadi et al., 1997). Despite the coded similarities, the mechanism which generates variations in the expression of the *MS vlhA* gene is very different to the one controlling the repertoire of the MG *vlhA* gene (Noormohammadi, 2000). In the *MS* genome, there is only one transcription - and translation - competent *vlhA* gene. However, as long as there is a single copy of the promoter and the first 408 bp of the coding sequence, there are multiple variants of the region coding the 3' end of the gene. These pseudo-genes are probably concentrated in a limited region of the genome as a tandem and apparently control the antigen variations in *MS* through expressions of multiple gene conversion (Allen et al., 2005).

In general, the process of subtyping the microbial isolates into strains is of great epidemiological significance for the identification of outbreaks,

determining the source of infection, especially for recognising the virulent strains of the organism and monitoring vaccination programmes (Olive & Bean, 1999). The methods used for differentiating the strains should have high distinguishing potential and be able to differentiate isolates from the same source. The molecular techniques used to identify the *Mycoplasma* strains are restriction fragment length polymorphisms (RFLP) of DNA (Kleven et al., 1988; Khan & Yamamoto, 1989), amplified fragment length polymorphism (AFLP) (Hong et al., 2005), pulsed-field gel electrophoresis (PFGE) (Marois et al., 2001), DNA and ribosomal RNA gene probes (Yogev et al., 1988), and PCR with strain-specific primers (Nascimento et al., 1993). The most commonly-used method for differentiation of strains is random amplified polymorphic DNA (RAPD) or arbitrarily primed PCR analysis (Charlton et al., 1999). The RAPD method is sensitive and fast, and is used to identify vaccinal strains in vaccinated flocks and epizootiologic studies. Progress in the molecular biology of mycoplasmoses over the last few years has led to the discovery of some surface antigens including *MS VlhA*. The DNA sequences of these genes could be used in the molecular epidemiology of *MS* (Raviv, 2007).

There are some significant variations among the isolates and their ability to cause illness, and there is no clinical expression of disease in many of the isolates. The *MS* isolates are more suitable for causing airosacculitis, and those from the synovial fluid are more likely to produce synovitis (Kleven, 1975).

In Giemsa stained preparations, the *MS* cells appear to have a pleomorphic coccoid shape and a size of approximately 0.2 µm in diameter. Colonies on a hard surface appear raised, round, with or without a centre. They vary from 1 to 3 mm in diameter depending on the available quantity, the medium and the age of the culture. Their resistance to disinfectants is probably similar to that seen in other mycoplasmas. *MS* is not resistant at temperatures higher than 39°C and pH below 6.8. In frozen materials, it may survive for a long time, but its titer is reduced. At -70°C it dies after 7 years, while at -20°C after two (Kleven & Ferguson-Noel, 2008).

EPIDEMIOLOGY

Chickens, turkeys and guinea fowl are natural hosts for *MS*, yet susceptibility has also been observed in geese, pigeons, pheasants and Japanese quails. *MS* is spread in most poultry-producing countries and the infection is often encountered in farms with mixed-age commercial egg-layer flocks. Transmission may be trans-ovarian or lateral, through airborne route or direct contact. Lateral transmission is carried out through the conjunctiva or the upper respiratory tract, and usually 100% of the birds become infected. Vertical transmission plays the primary role in the spread of *MS* among birds. Following infection, the birds are persistently infected with *MS* and remain carriers their entire lives (Raviv, 2007). Nevertheless, many flocks hatched from infected breeders remain infection-free.

There have been reports of a 78.6% seropositivity rate against *MS* among commercial egg-layer hens in East England, and 87% in South California. The high prevalence and persistence in these cases is explained by the popular practice of breeding mixed-age flocks and the low biosecurity standards in this sector (Gole et al., 2012).

Gole's team succeeded in proving experimentally the effect of *MS* on the eggshell hardness in broiler breeders, which contradicts the findings of certain researchers who maintain that there is no such effect. Flocks infected with *M. synoviae* represent a potential risk to other categories of birds. A case in the Netherlands seems to show a MS strain in association with an EAA formation, as well as a potential synergism between MS and infectious bronchitis (Feberwee et al. 2009 a, b).

CLINICAL SIGNS AND PATHOLOGY

The economic significance of *MS* infection in egg-layers has usually been considered low due to the small or negligible effect on egg production rates and quality (Branton et al., 1997). In experimental studies with challenge infection using the *MS* K3344 strain, researchers assumed that it could serve as a primary factor in the widespread and most commonly encountered *E. coli* peritonitis syndrome (Raviv, 2007).

Clinical and morphological signs of *MS* in broilers chickens

The incubation period varies significantly, depending on the route of infection, the virulence and counts of the organisms, the presence of predisposing factors and the host's susceptibility. After an intra-sinus inoculation, the incubation period is 7–14 days, after conjunctival infection it is 20 days, and after contact infection 11–21 days (Stripkovits, L. & I. Kempf, 1996).

Synovitis

Clinically, symptoms include retarded growth, joint oedema, lameness, ruffled feathers, as well as reduction of spontaneous locomotive activity and frequent lying down. Most commonly affected are the tibio-tarsal and tarso-metatarsal joints. *MS* is frequently etiologically related to sternal bursites. The morbidity and death rates are moderate, under 10%. Young chickens at the age of 4-12 weeks and turkey poults at the age of 10-12 weeks are susceptible. Synovitisis encountered all year round, but are prevalent during cold, humid seasons or when the litter is wet.

Affected birds get progressively exhausted. When the joints and tendon sheaths are open, a serofibrinous exudate is most commonly observed. *MS* shows a certain tropism to synovial structures as joints and tendon sheaths. *MS* infections should be differentiated from staphylococcal infections, reoviral arthritis and RGT (see RGT).

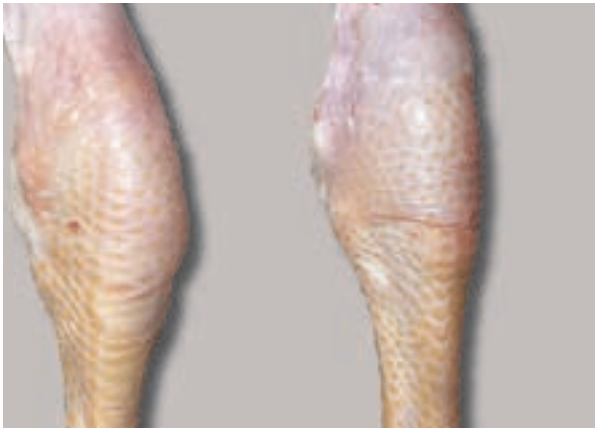

Fig.1
Moderate bilateral oedema of the tibio-tarsal joints of a 14-week-old broiler breeder.

Fig.2
Marked unilateral oedema of the tarso-metatarsal joints of a turkey at 8 weeks of age.

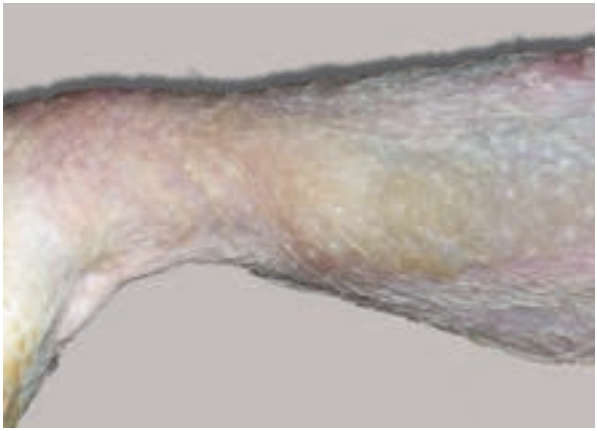

Fig.3
Subcutaneous transparency of gelatinous thick exudate in the synovial sheath, proximal to the tibio-tarsal joint.

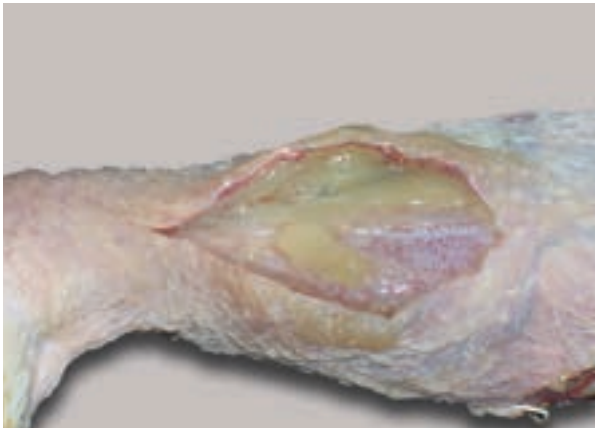

Fig.4
The image from Fig. 3 after removing the skin and opening the synovial sheah.

Fig.5

Advanced stage of MS synovitis, accumulation of fibrinous exudate in the synovial sheath.

Respiratory form

In addition to a symptomatic respiratory infections, MS is capable of inducing airosacculitis. The lesions of the respiratory tract are similar to those induced by Mycoplasma gallisepticum, yet with milder expression (Jordan, 1979).

Egg apical abnormalities

The term egg apical abnormalities (EAA), also known as "glass-top eggs" represents a specific eggshell lesion characterised by a well demarcated region of thin and soft shell at the tip of the affected egg. This anomaly makes the eggs more fragile. Hypothetically, it is assumed that some *MS* strains have a reproductive tract tropism and may form colonies under certain circumstances. The result is the production of eggs with abnormal shells. Even though the lesion is highly distinctive, it should not be accepted as pathognomonic for a certain pathogen or a combination of pathogens. EAA is a relatively new condition.

Abnormal egg yields may vary between 1–2% and 25% in a flock affected by EAA. The problem may persist until the end of the flocks' production period. Due to economic concerns, the flock may have to be culled prematurely. The affected eggs exhibit a specific

shell defect at the sharper tip, which is thin, soft and easily breakable, sharply delineated from the rest of the shell, which appears to be normal. No lesions are found in tissues from the oviduct when studied under the microscope.

Fig.6
EAA. The egg poles at the sharper tip are discoloured, deformed and fragile.

DIAGNOSIS

Diagnosis is based on epidemiological data, clinical signs of the disease, analysis of macro- and microscopic lesions, serology and/or isolation and identification of mycoplasmas. The etiological agent may be found in fragments of affected organs (lung, trachea, air sacs), such as the infraorbital sinuses or synovial exudate. The most commonly used serological tests are serum plate agglutination (SPA), hemagglutination inhibition (HI), and enzyme-linked immunosorbent assay (ELISA), followed by isolation and identification of mycoplasmas. Tracheal and cloacal swab samples are used in the isolation of the agent

through polymerase chain reaction (PCR), (Luciano et al., 2011). SPA, HI, and ELISA exhibit weak statistical correlation in the diagnostics of MS. The same authors recommended using these diagnostic tests (SPA, HI, and ELISA) as screening tools in monitoring programmes for detecting mycoplasmoses in breeder flocks. Positive results must be confirmed through isolation of the organism using the traditional microbiological methods or biomolecular analysis (PCR). It is difficult to isolate MS from synovial, joint or bursal lesions in chronically affected birds.

DIFFERENTIAL DIAGNOSIS

Synovial *MS* infections should be differentiated from staphylococcal infections, reoviral arthritis and rupture of the gastrocnemius tendon.
The respiratory form should mainly be differentiated from MG, Swollen head syndrome and IB infection.

Differential Diagnosis of EAA

The occurrence of abnormal eggs raises the possibility of EAA. Post-mortem analysis is recommended, as well as laboratory testing to identify the cause(s). There are many infectious and non-infectious conditions that may cause poor-quality eggshells, and EAA must be differentiated from them. Among the infectious diseases, potential causes include IBV, which affects the second and last third of the oviduct mucosa, and causes the occurrence of watery egg albumen; EDS'76 is also a possibility, caused by an adenovirus which damages primarily the last third of the oviduct mucosa, leading to eggs with soft or irregularly shaped shells or even eggs without shells. In this case, there is a sharp drop in egg-laying capacity without changes in the flock's health status. In infectious laryngotracheitis (ILT) and infectious avian encephalomyelitis (IAE) eggs with rough ("sandpaper") shells appear. In Newcastle Disease (ND) and Avian Influenza (AI) there are also eggs with poor shell quality.
Non-infectious factors that could affect the quality of eggshell formation include some mycotoxins such as ochratoxin; chemical

toxins such as organochlorine contaminants (shells with thin cracks, jagged, breakages at the pole); and the birds' macronutrient status – calcium / phosphorus / vitamin D. Defects in egg shell formation may also be caused by excessive dietary phosphorus or sodium.

PREVENTION AND CONTROL

The primary route of infection transmission is vertical. In this respect, efforts should be directed towards MS-free breeder flocks. Antibiotic treatment of the breeders does not eliminate *MS* effectively, even though the extent of transmission through the eggs can be reduced (Kleven & Ferguson-Noel, 2008).

Eradication of the Mycoplasma infection requires changes to hygiene and management practices in order to reduce shedding of mycoplasma into the environment. This includes the afore mentioned antibiotic treatment of breeder flocks and their hatching eggs.

Vaccination

MS does not induce a strong immune response. A live vaccinal strain MS-H was selected from a field Australian isolate. The same strain colonises the trachea of the chickens and provokes serum antibodies 3 weeks after instillation into the eye. Its efficacy and safety were tested in both laboratory and field conditions. This vaccine has found widespread application in Australia (Kleven & Ferguson-Noel, 2008).

After experimental testing of a live vaccine (Vaxsafe†MS; Bioproperties Pty Ltd, Ringwood, Victoria, Australia) against Mycoplasma synoviae inducing EAA, it was concluded that vaccination does not prevent the problem entirely, yet can reduce it (by approximately 50%) (Feberwee et al., 2009a).

TREATMENT

MS exhibits in vitro sensitivity towards some antibiotics – tetracycline, chlortetracycline, oxytetraccline, enrofloxacin, lincomycin, etc. In general, the application of an adequate medication is a suitable method of preventing airosacculites and synovites, but is not effective for treatment of pre-existing lesions. Airosacculitis in broilers can be controlled to a great extent through water-soluble lincomycin-spectinomycin or tiamulin. Prolonged medication of feed with chlortetracycline has beneficial effect on the prevention of synovitis in chickens (Kleven & Ferguson-Noel, 2008).

Various antimicrobials with anti-mycoplasma activity and vitamin D supplementation were applied to one affected flock with little effect on the spread of EAA eggs. Treatment with tylosin improved the quality of the eggshells, reduced economic losses and increased the eggs' weight throughout the productive cycle (Catania et al., 2010).

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