# Respiratory diseases of turkeys – part two

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Mycoplasmas have affected poultry production for many years and effective control of mycoplasma infection has been a fundamental stepping stone to improved performance and productivity. However, infections appear to be making a comeback.

Numerous species of mycoplasmas have been isolated from avian sources. Two species are recognised as predominantly pathogenic to chickens and turkeys. Mycoplasma gallisepticum (MG) affects the respiratory system and is referred to as chronic respiratory disease (CRD) in chickens, and infectious sinusitis in turkeys.

Mycoplasma synoviae (MS) may cause either respiratory diseases and/or joint diseases. Two additional species are known to be pathogenic to turkeys. Mycoplasma meleagridis (MM) causes airsacculitis, and Mycoplasma iowae (MI) causes decreases in hatchability.

Mycoplasmas are the smallest self replicating procaryotic organisms. They have no cell wall but are bounded by a triple layered plasma membrane which is composed mainly of lipids and proteins.

***Infectious sinsusitis – mycoplasma.***

## Environment sensitive

The genus Mycoplasma belongs to the family Mycoplasmataceae, order Mycoplasmatales of the class Mollicutes.

Mycoplasmas are sensitive in the environment and susceptible to the most commonly used chemical disinfectants.

The viability of mycoplasmas outside the host is of short duration – 2-8 days at 5-10°C. Sunlight kills the organism in 20-30 minutes. They remain stable in faeces at 20°C for three days, in hatchery fluff for five days and at -20°C for several months.

Mycoplasma can survive in the human nasal passage for 24 hours, on human hair for three days, and on feathers for 2-4 days. They may exist for very long periods in the respiratory tract of infected birds and these apparently healthy carriers are essential for mycoplasma survival in poultry populations.

The disease spreads from flock to flock by vertical transmission through infected eggs. Infected progeny then transmit the agent horizontally either by direct bird to bird contact or by indirect contact through contaminated feed, water and equipment.

Concerning vertical transmission, hens which become infected before the onset of laying tend to egg transmit at a lower rate than hens initially infected during egg production. Generally egg transmission is intermittent and the rate is variable (1-10%) and very low. The spread of infection from bird to bird within one pen is usually rapid but it is rarely transmitted from one pen to another.

However, in continuous production complexes (multiple-age) with chronic apparent healthy carriers the spread of infection is difficult to control since the cycle of infection can not be broken without complete depopulation.

The agent can also be transmitted by other species of birds as well as mechanically by other animals and man. Bradbury (1999) reported on the problems related to re-emergence of mycoplasma infections. In the past mycoplasmas appeared to have a restricted host range, which should help to limit their lateral spread.

This does not seem to be true for MG or MS, both of which have been found in a number of avian hosts. A widespread epidemic of MG infection also occurred in North American finches.

In addition, MM was isolated from raptors in Germany and MI from chickens and from wild and exotic birds.The clinical signs and the course of the disease are influenced by several factors such as the presence of concurrent micro-organisms (TRT, influenza, reo and E. coli) and/or improper management (increased dust and ammonia levels in the environment).

The clinical manifestation due to Mycoplasma gallisepticum (MG) may include drops in feed consumption, coughing, sneezing, râles, ocular and nasal discharge and swelling of sinuses. In some cases, sinusitis may be absent and only rhinitis, tracheitis and airsacculitis could be found accompanied by a fibrinous pneumonia in some cases.

## Mcyoplasma synoviae

Mycoplasma synoviae (MS) has the affinity for synovial membranes and may infect the membranes of joints resulting in swelling of the joints followed by lameness.

Hocks, footpads, wing joints and the sternal bursa are most frequently involved. Affected birds are not able to move and lose or fail to gain weight. MS may also cause respiratory disease and airsac lesions similar to MG.

The role of MS as a primary pathogen in both respiratory and locomotory disease of turkeys is less clear and other factors may be involved and responsible for the onset of clinical signs.

Mycoplasma meleagridis (MM) causes embryo deaths, sinusitis, stunting, airsacculitis, and occasionally bone defects as well as swollen hock joints. Mycoplasma iowae (MI) causes mortality of turkey embryos

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and some strains may cause inflammation of the joints, but the organism appears to be rarely encountered since its eradication by the primary breeders.

Diagnosis of mycoplasma on the basis of clinical features and pathological lesions is often difficult, since these signs may be confused with other infectious diseases. Proof of infection, therefore, must be confirmed by laboratory diagnosis either by direct detection, direct isolation or indirectly using serological methods.

## Acute stages of infection

During the acute stages of infection the population of mycoplasma in the respiratory tract is very high. In such cases 5-10 tracheal or chonal cleft cotton swabs are sufficient for mycoplasma isolation.

In chronic cases however, a high number of samples is essential. In dead birds culture of lesions should be carried out. For culturing embryonated eggs, samples of yolk and yolk membrane should be included.

The medium developed by Frey et al. (1968) is widely used for isolation of MG and M. synoviae. Specimens should be cultured within 24 hours. With MM and MI, primary isolation on agar plates may be more successful than using broth culture.

The isolation is accompanied by several disadvantages:

- The organism is relatively fastidious and slow growing and culturing requires about 21-28 days confirming a negative result.
- The growth of mycoplasmas may be inhibited by damage during sample transport to laboratory or by growth inhibitors in the medium.
- In some flocks several different mycoplasmas (non-pathogenic) can be grown from a single sample and compete with the growth of a pathogenic one.

To avoid such difficulties MG and MS DNA probe test kits have been developed and are now commercially available.

## DNA probe test kits

The test is highly sensitive and specific and is able to detect small amount of mycoplasmas even in long term frozen samples within eight hours.

The cost is comparable to isolation procedures. In addition, several PCR based tests have also been developed for MG and are cited by Kempf (1998) including a multiplex PCR, which is designed to detect all four avian mycoplasma pathogens.

Furthermore, a manual published by Lauerman (1998) contains a validated PCR assay for avian mycoplasmas.

For serological examinations the most commonly used initial screening test is the rapid serum plate agglutination test (SPA). The test is based on the use of specific stained antigen for MG, MS and MM that are commercially available. The test is quick, inexpensive and highly sensitive, but there are also variations between batches in sensitivity and specificity.

Serum plate agglutination suspected reactors generally must be confirmed by other tests such as a haemagglution inhibition test (HI) using fresh culture antigen. The corresponding titres are 1:40 and 1:80 or above respectively.

The HI test appears to be more specific than SPA, but still shows wide variations in results due to lack of uniformity between antigen preparations and that some isolates fail to agglutinate red blood cells and do not stimulate the production of HI antibody. ELISA kits have also been developed for detection of antibodies against MG, MS, and MM. These kits are commercially available.

ELISA is sensitive and specific but can also be prone to false positive and negative results. Generally, significant antigenic variability among strains also exists, which could affect the sensitivity of serological tests. In all cases confirmation of positive cases can be carried out only by retesting the birds after about a month, or by culture.

### Treatment and control

Several drugs have been found useful for reducing clinical signs and shedding in infected flocks. However, no antibiotic regardless of dosage or length of treatment can eliminate the infection in birds and hatching eggs. Among the more common antibiotics are tylosin, spiramycin, tetracycline, quinolones (enrofloxacin, flumequin), spectinomycin and lincomycin.

The drugs can be administered by numerous routes (Injection, feed, water). Treatment is able to reduce the losses, but relapses may occur when treatment is discontinued. Since the isolation of the causative agent is difficult and time consuming, currently little is known about the susceptibility of recent isolates.

Eradication of mycoplasma in breeder flocks through testing and slaughter is the preferred method to clean the production chain from the top and to prevent mycoplasma introduction through primary and commercial breeder flocks. However, in places with intensive continuous poultry production and in valuable pedigree lines it has been determined that this method is too expensive and impractical.

Hatching egg treatments with antibiotics for the control of egg transmitted bacterial pathogens has been widely investigated and seems to be of great value. Different methods of egg treatment have been used such as egg dipping in antibiotics using pressure differential dipping or temperature differential dipping. These methods greatly reduce the mycoplasma egg transmission, but do not always completely eliminate it.

Dipping solutions can become excessively contaminated with resistant micro-organisms such as pseudomonas and organic

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material. To prevent bacterial contamination of the solution filtering with subsequent cool storage and/or addition of disinfectants is the most effective method. Thorough and continuous bacteriological monitoring of dip solution is also required.

The concentration of the antibiotics must be examined regularly and renewed routinely. By using enrofloxacin the pH value of the dipping solution can be corrected during storage.

The use of egg dipping in antimicrobials should be critically evaluated, because of the irregular uptake of dip solution, uneven distribution of active substance in the egg compartments and lack of standardisation in dipping technique.

Additionally, it is known that different disinfectants used for washing can influence negatively the antibiotic uptake of hatching eggs. Therefore, it is recommended that the compatibility of different disinfectants used for egg washing and/or used in dipping solution has to be examined before application.

## Irregular uptake

As the uptake of active substance by the hatching egg can be very irregular during dipping, individual egg injection with accurate delivery of the proper dose is preferred in elite and grandparent stock breeding. Automated systems for in ovo drug disposition before hatch are being developed.

Vaccination against MG infection using inactivated culture was attempted in the early 1950s without success until oil emulsion inactivated vaccine 'bacterins' were introduced.

The inactivated bacterins came into widespread use in the early 1980s and were primarily used in commercial layer flocks and now are used in broiler breeder flocks in many areas worldwide. The bacterins provide good protection against egg production losses. In vaccinated flocks feed conversion improvements and reductions of medication costs have been demonstrated. In vaccinated breeder flocks a drastic reduction of egg transmission has also been observed.

The lag period between infection and egg shedding in vaccinated flocks is longer, and this period could be prolonged by revaccination of the birds during production.

## Vaccine disadvantages

However, inactivated vaccines have some disadvantages – they are expensive; they must be applied by injection which leads to higher labour costs, and they do not provide optimal protection against infection and tracheal colonisation by field strains.

The live vaccine is less expensive and in most cases can be mass applied.

At the end of the 1970s a live attenuated vaccine using F-strain was successfully used to protect chickens against MG infection.

This strain, however, is virulent for turkeys. A live MG vaccine called 6/85 strain has been introduced. It is apparently less pathogenic for both chickens and turkeys providing a significant protection against airsacculitis and egg losses.

Also a live vaccine based on a temperature sensitive (TS-11) mutant strain of MG has been developed and is widely used in Australia and licensed in some other countries.

MG vaccines have had less use in turkeys. The F strain is too pathogenic for consideration in turkeys, but 6/85 or TS-11 strains may have potential use under very limited circumstances. In one vaccination trial administration of 6/85 or TS-11 did not result in respiratory signs or lesions in turkeys. There was little or no measurable resistance against airsacculitis after heavy aerosol challenge, but there was some protection observed against lesions in the upper respiratory tract.

According to Kleven (2004) the TS-11 strain appears to have limited ability to infect turkeys. On the other hand, vaccine based on the 6/85 strain has been used in the field to vaccinate turkeys, with varying results. There has been relatively little work on MS vaccines.

There has been one MS bacterin licensed in the USA, but it apparently has had little field use. A temperature sensitive MS strain based vaccine has been licensed in Australia, however, no data is available about its effects in turkeys.

## Conclusion

As respiratory diseases of turkeys are mostly associated with severe economic losses, early recognition and monitoring programmes are essential in managing the infections.

Generally, therapy or vaccination alone is of little value, unless they are accompanied with improvements in all aspects of management and biosecurity.

Biosecurity is the cheapest, most effective means of disease control available and no disease prevention programme will work without it.

Since the success of any control programme depends on the hygiene practices of the staff, it is essential to incorporate education programmes about micro-organisms and their modes of transmission, as well as awareness of the reasons behind such control programmes for all people involved throughout the poultry production chain.

In the long term, development of turkey lines that are genetically resistant to some pathogens should be progressed and further attention must be paid to the development of efficient vaccines against bacterial infections to reduce the use of antibiotics. ■

*References are available from the author on request.*