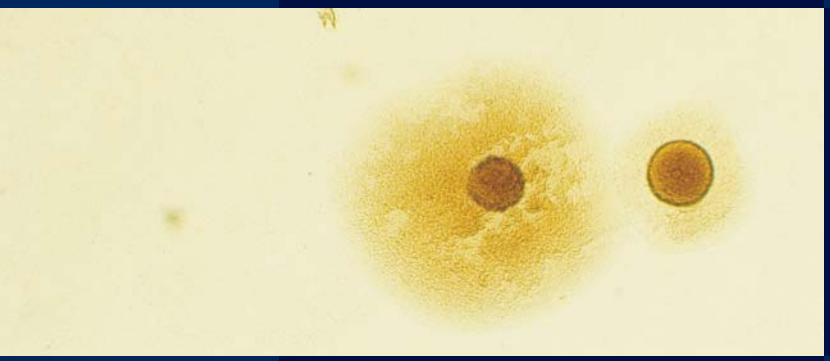

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Ross Tech

AVIAN MYCOPLASMOSIS

Avian Mycoplasmosis

Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) are bacteria without cell walls that infect chickens and other birds and can, in some circumstances, cause disease. Some infections may appear clinically silent but probably still involve a production penalty, e.g. a decrease in the total number of eggs/chicks or decreased broiler performance.

MG is usually more likely to cause disease and therefore a greater production penalty than MS. However, there is wide variation in characteristics within each species and between strains, including virulence and kinetics of the serological response. The expression of disease (and serological response) is modulated by many other factors including management, environment and immunity.

When Mycoplasma causes clinical disease it is usually respiratory disease. In uncomplicated infections this could be seen grossly as airsacculitis, *see figure 1.* Joint disease, tracheitis, swollen sinuses and conjunctivitis are also sometimes seen, *see figure 2.* MS and MG infection in broilers will increase condemnations in the slaughterhouse. In parent stock, infection during lay will often cause decreases in egg production, airsacculitis in embryos and increase late dead embryos (airsacculitis may be seen in "pipped" embryos). In commercial layers, MG infection without clinical signs is estimated to decrease egg production by 10 to 20 eggs per hen housed.

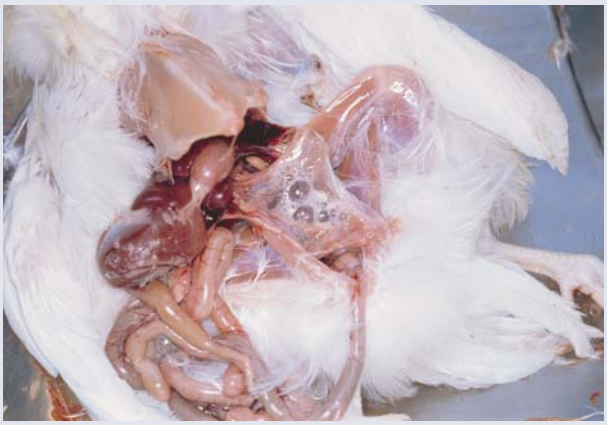

***Figure 1** - Experimental airsacculitis 10 days after simultaneous infection with MS and infectious bronchitis virus.*

MG and MS can both exacerbate respiratory diseases, interacting with respiratory viruses, dust, ammonia, and opportunistic bacteria (e.g. *E.coli* and similar bacteria). In this case chronic respiratory disease (CRD) is triggered and mortality may be elevated. Good air quality and the implementation of minimum ventilation rates have a protective effect, *see figure 3.*

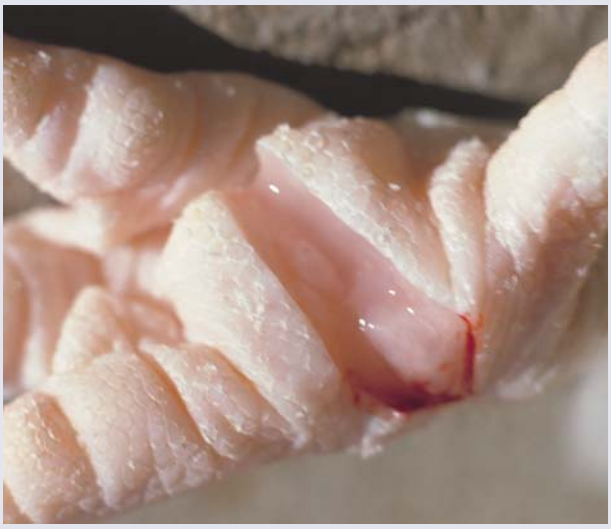

***Figure 2** - Field case of MS associated tenosynovitis in a broiler parent stock. Some strains of MS, and less frequently MG, are more likely to cause leg problems than others.*

MG is officially controlled and/or monitored in many countries, but MS control is mostly the policy of an individual company. Primary Breeders control MS, but this control may cease further down the production chain. In commercial layers there is usually no control of MS infection as most birds are housed during lay on multi-age sites, which therefore remain a reservoir for infection of other poultry facilities. This variable approach to the control of MS in poultry populations is one of the main problems in broiler parent stock as large reservoirs of infection may exist in adjacent layer facilities.

Since the discovery of the importance of MG in respiratory disease of chickens there have been many attempts to produce chickens without Mycoplasma infections. These are complicated by vertical transmission, the inability of traditional diagnostic tests to identify all infected flocks, the lack of clinical signs in some infections, the chronic nature of the infection state and the inability of antibiotics to reliably eliminate infection.

The lack of a cell wall by mycoplasmas means that they are very fragile and die rapidly outside the host bird. Antibiotics that are active against cell wall production, e.g. penicillins and cephalosporins, are ineffective. Mycoplasmas are sensitive to tetracyclines, tylosin, tiamulin, quinolones (enrofloxacin) and tilmicosin but most of these are bacteriostatic rather than bacteriosidal. They can be given variously by injection, in water or in feed. Acquired antibiotic resistance has been described to most of these drugs. Sulpha drugs have limited activity. Generally antibiotic administration will not eliminate Mycoplasma infection but if effective it will decrease clinical signs and Mycoplasma population numbers. Once infected a flock must be considered infected for life and therefore a risk to other uninfected flocks.

To prevent vertical transmission, eradication of MG and MS has been achieved at the Primary Breeder level and this is monitored continuously. The sourcing of Mycoplasma free stock is the first step in Mycoplasma control.

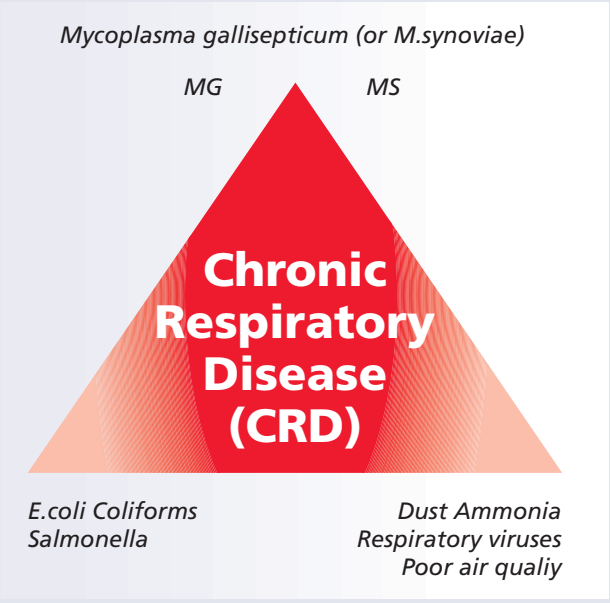

Figure 3 *- Interaction of factors involved in the development of Chronic respiratory disease. Such a model suggests that control of Mycoplasma will help decrease mortality and other losses due to CRD but also suggests that control of other contributing factors can have an effect.*

If biosecurity is good enough it may be possible to prevent horizontal infection. The main causes of Mycoplasma infection in flocks are via aerosol infection, contact with other birds, and mechanical transportation by humans or a combination of any or all these. Distance is the greatest protection against aerosol infection. MS appears to be able to transfer between flocks over greater distances than MG. Other birds closely related to the chicken, including turkeys, guinea fowl, partridges, pheasants, quail, ducks, etc., are the greatest risk to poultry for Mycoplasma transmission. Mechanical transmission is also possible; humans can carry the avian mycoplasmas in their noses and on hair for up to three days. Showering and quarantine periods can help prevent this.

Management strategies such as spiking and thinning can spread Mycoplasma within an operation. Precautions and testing must be undertaken to reduce the risk of these procedures spreading the infection.

Diagnosis by demonstration of the organism (Gold standard)

Culture and PCR testing are the best confirmatory tests available. Swabs taken from the trachea or choanal cleft are placed into Mycoplasma media and sent to the laboratory. PCR testing can also be completed on this type of sample or on air dried swabs, *see figure 4.*

Figure 4 *- Dry swab PCR sampling*

There are some mycoplasmas of poultry that are considered non-pathogenic, therefore species identification after culture is essential to assess the problem. This is done by using specific antibodies. To culture MS there must be extra NAD in the media.

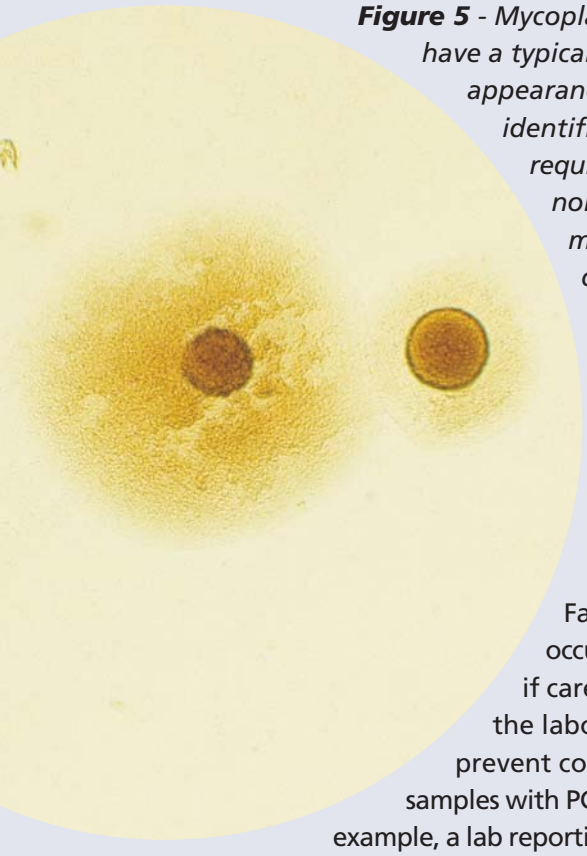

Figure 5 *- Mycoplasma colonies have a typical fried egg appearance and species identification is required as there are non pathogenic mycoplasmas that can also infect chickens. Mycoplasma culture is specialised and not readily available everywhere.*

False positives can occur with PCR tests if care is not taken in the laboratory to prevent contamination of samples with PCR products. For example, a lab reporting PCR positives from samples collected from day old chicks, where the source flock has been routinely tested negative by serology, is likely to be incorrect. Suspicion should be cast on the PCR test protocols. A recent ring test reported 5 out of 7 labs with false positives for MG & MS PCR's. An example of PCR results is shown in figure 6.

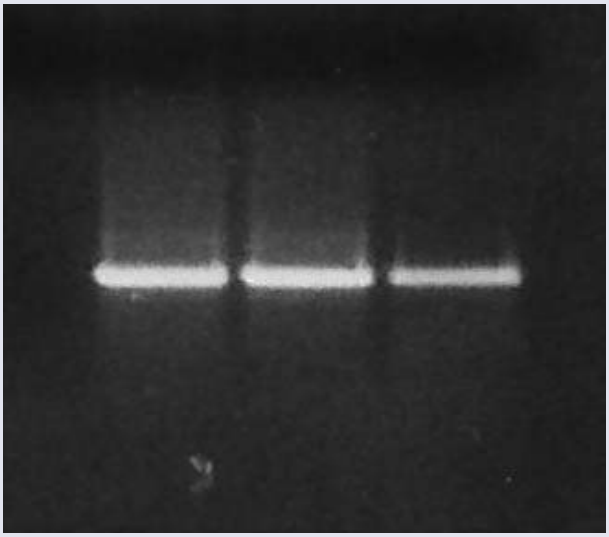

Figure 6 *- Mycoplasma PCR bands indicate that specific Mycoplasma was in the sample. Many different Mycoplasma PCR's are available but if they are not species specific the results can not be interpreted from clinical samples.*

Monitoring by serology

Routine monitoring of flocks for MG and MS infection is usually by the RSA, (Rapid Sera Agglutination also known as the Plate test, SPA) or ELISA test. Testing is usually recommended to be every 3 weeks in high risk areas. This allows eggs to be pulled from the incubator if there is a problem. No sampling should be done for 3 weeks after a killed vaccine is administered as this is a common cause of false reactions. RSA testing should only be done on sera less than 72 hours old and care in handling the samples should also be taken to minimize false reactions. Heat treatment of sera (56°C for 30mins) is commonly practised on positively reacting sera and titration of sera for interpretation of the tests. Both MS and MG should be tested for, as a recent MS infection will cause false MG reactions. On the appearance of a small number of reactors in the RSA test the flock should be placed in quarantine and retested with serology or by PCR or culture to confirm the result. On the appearance of a large number of reactors (>15% of samples after heat treatment) the flock is more likely to be truly infected and appropriate isolation steps should be taken as well as confirmation testing.

Yolk samples from eggs can be tested in ELISA format tests and may be useful, especially if access to the parent flock is not possible or is a problem from a biosecurity point of view. Testing day old chick (DOC) sera is problematic, but all samples should be heat treated before testing or false positive rates can typically become more than 15%.

Do not rely on test of DOC sera. False positive results are very common.

The RSA test needs extensive quality control to be performed properly, *see figure 7.* Titration of antigens with a standard lyophilized positive (and negative) serum is usually undertaken. New batches of antigen should be tested to make sure that the specificity is appropriate for your needs. Return unsuitable batches to the manufacturer.

Vaccination

There are two main groups of commercial vaccines available against Mycoplasma – live and dead. In practice these have different applications. Dead vaccines can be used to decrease clinical signs associated with Mycoplasma infection and greatly reduce vertical transmission, but they do not stop infection of the birds with wild type mycoplasmas. This makes vaccinated stock a potential hazard to unvaccinated stock. For instance, parent stock vaccinated to stop vertical transmission are a significant risk to broiler stock through horizontal infection. One advantage of killed vaccines may be that they can be given to a positive flock and may provide some beneficial effects. Mycoplasma infection in parent stock is often clinically silent, therefore no advantage may be seen in vaccinated birds; indeed controlled exposure during rearing was a method of natural vaccination suggested in the 1960s.

Live vaccines have to be given before the wild strain mycoplasmas infect a significant proportion of a flock. There is some evidence that they will exclude the wild strain infections and therefore reduce the risk of creating a reservoir infection in vaccinated flocks. In addition this will also decrease the clinical signs and vertical transmission of the infection. In designing vaccination programmes with live Mycoplasma vaccines it is necessary to know the usual epidemiology of Mycoplasma infections in a farming operation and then introduce the vaccine at least one month before wild challenge is expected. No medication with antimycoplasmal activity can be given immediately before, during or for some period after the live Mycoplasma vaccination.

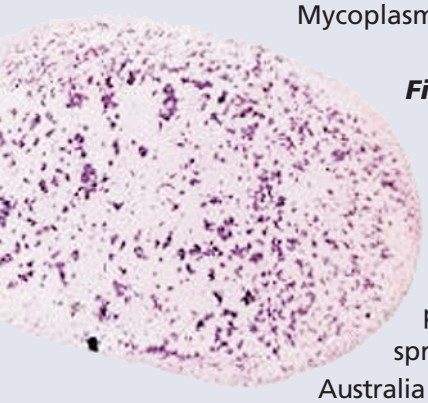

Figure 7 *- 4+ Agglutination*

For MG there are two live vaccines being used extensively in the world: *6/85* (Intervet), a lyophilized product given as a coarse spray and *ts-11* (Bioproperties Australia and under licence elsewhere) delivered frozen on dry ice and given by eyedrop. *MS-H* (Bioproperties Australia), delivered frozen on dry ice, is the only live MS vaccine available.

In areas where Mycoplasma breaks are common these vaccines are used on all flocks routinely. Production managers then know that there will be no drops due to Mycoplasma during production.

Homologous antigens in ELISA formats may improve detection of vaccine responses. Typically *6/85* produces little in the way of antibody response in the RSA test. The response to *ts-11* is variable but *MS-H* produces a regular response. These responses will be seen as maternal antibody in day old chicks but should disappear within three weeks.

Cleanout and disinfection

Mycoplasmas are very fragile organisms, rapidly dying if away from the host unless they are protected by moisture and organic material. Cleaning of the house needs to be thorough, using physical removal, then detergents and a terminal disinfection. Care needs to be taken in disposal of litter so as not to contaminate other farms. Litter from contaminated flocks should be stacked and left for 3 weeks before disposal.

Sustainable broiler production systems

Broiler production developed seriously as an industry when grain surpluses became available in the 1950s. Over the last fifty years the technology to maximize production and profitability has developed, with constant advances in genetics, management and disease control. Modern biosecurity can be used as a tool to control Mycoplasma infections and minimize Chronic Respiratory Disease in broiler operations.

The original broiler production systems developed throughout the world were typically large sites with parent stock farms and broiler farms all in close proximity. Economies of scale and utilization of labour and equipment were the drivers of this original design.

Unfortunately such systems are easily exploited by chicken pathogens and broiler health and performance are often compromised. Technically this is commonly known as CRD (Chronic Respiratory Disease) or sometimes called

Colibacillosis. These sites may be known as "Chicken Sick Farms". Typically these operations perform well for a variable period of time before there is an introduction or build up of pathogens with clinical signs and mortality usually increasing at about 28 days of age and continuing until slaughter. Good performance is never achieved again on the site. As the build up of pathgens increases the mortality may become greater and/or start earlier. More antibiotics are tried and sometimes performance and mortality will improve for short periods, but eventually performance deteriorates again. The pathogens may arrive vertically or horizontally.

Extreme environmental temperatures make the design of effective ventilation systems difficult. Minimum ventilation rates must be defined and adjusted, in relation to the bodyweight of the birds, and then applied.

Clinical investigations will reveal:

1) *E.coli* and similar bacteria progressively become more resistant to antibiotics paralleling exposure to these chemicals. ORT *(Ornithobacterium rhinotraceale)* may also be found.
2) *Mycoplasma gallisepticum* or perhaps *Mycoplasma synoviae* are present and these too may become resistant to antibiotics.
3) Various respiratory viruses including Lentogenic Newcastle Disease viruses (NDV), Infectious Bronchitis virus (IBV), Avian Pneumovirus (APV, or TRT virus) and sometimes ILT (Infectious Laryngotracheitis) will be found. Some of these may be the progeny of vaccine strains being used.
4) Infectious Bursal Disease (IBD) virus or Mareks may also play a part.
5) Other factors including dust, inappropriate humidity, poor air quality (ammonia and other gases due to minimum ventilation rates being inadequate), cold or heat stress and unsanitary conditions may also be present.

Considering the epidemiology and pathogenesis of CRD, there is initial respiratory damage by respiratory viruses or poor air quality. Mycoplasma infection will aggravate this further if it is also present. As this progresses *E.coli* superinfection occurs and death results. In addition the remainder of the flock may be uneven.

Often there will not be a requirement for a large scale clinical work up on such cases with MIC testing (antibiotic sensitivity testing) of bacteria and Mycoplasma, and PCR detection of viruses, etc. Simple biosecurity will stop the perpetuation of the bacteria by stopping continual horizontal re-infection. Biosecurity is the simple separation of the bird from the pathogen, be it a bacteria, virus, or parasite. 'All in- all out' protocol refers to pathogens as well as birds unless they are very resistant to cleaning (for example Coccidiosis, IBD or Mareks), and the removal of the birds to the slaughterhouse will also remove the Mycoplasma and respiratory viruses as these pathogens do not survive for long away from the host. Having only one generation of birds on a site means that vaccine viruses cannot undergo bird to bird passage, reverting to virulence and thus challenge subsequent batches of birds. Control of the movement of humans, equipment, trucks, etc. will also control the movement of pathogens.

For those pathogens where their absolute movement cannot be controlled, we can use vaccination to help manage their disease producing potential.

Often the outcome of an infection is associated with the quantity and timing of challenge. A bird may find that being challenged later in its life causes fewer problems because its organ systems are more developed or immunity from vaccination may be induced. The risk of horizontal transmission for those pathogens spread by aerosol will be influenced by the size of the infected population, the distance between birds and the population size of the naive flock. Contact with other birds, through poultry or staff, is the biggest risk to the flock.

There is an economic cost of biosecurity, but systems based on biosecurity are sustainable. They do not rely on the routine use of antibiotic prophylaxis and therefore are not susceptible to failure due to the development of antibiotic resistance. Savings can be made in decreased medication costs, while the benefits are better quality production.

Mycoplasma control strategies

Strategy	Comments	Advantages	Disadvantages
Run Mycoplasma free.	Need good biosecurity. May be able to move from a positive status to negative status with live vaccination.	Low input costs.	Always worrying about flocks becoming infected. Higher capital costs.
Live vaccination of Parent Stock.	Must vaccinate stock before wild Mycoplasma challenge.	No clinical signs in parent stock and reduced danger.	Positive antibody and PCR status. May be incompatible with export. May limit therapeutic options around vaccination.
Killed vaccination of Parent Stock.		No clinical signs in parent stock. Only need to vaccinate before onset of lay.	Danger of silently infected Parent Stock being a source of infection for broilers.
Strategic medication.	Parent Stock and broilers may both need to be medicated.		High cost of antibiotic usage and potential future problem with development of antibiotic resistance.
Do nothing (or only control MG and not MS).	Uncompetitive performance.		High mortality and poor performance in broilers and increased condemnations.

Conclusion

- **Decide on strategy to control Mycoplasma, see table above.**
- **Run free of MG and MS**
  or
- **Consider live vaccination**

General biosecurity rules that protect against the transmission of Mycoplasma include:

1) Single age farms.
2) All in – all out housing.
3) Secure barrier round perimeter of farm with controlled access.
4) Wild bird proofing of facilities.
5) No staff should own poultry at home or have contact with other birds.
6) Shower on and shower off facilities on farms.
7) Visit clean flocks before infected flocks.
8) Visit youngest flocks first.
9) Hatch chicks from infected flocks separately.
10) Implement a monitoring programme, with regular testing, to define the status of the birds.
11) Farms should be at least 2km from other concentrations of poultry.
12) Planning feed and egg transport to minimise risk.

Acknowledgement: The pyramid diagram was suggested by the Late Robin Cumming, an early researcher into Mycoplasma in Australia and South Africa.

This information comes to you from the Technical Team of Aviagen. Although it is considered to be the best information available at the present time, the effect of using it cannot be guaranteed as performance can be affected substantially by many factors including flock management, health status, climatic conditions, etc.

Every attempt has been made to ensure the accuracy and relevance of the information presented. However, Aviagen accepts no liability for the consequences of using the information for the management of flocks. Data presented in this Ross Tech should not therefore be regarded as specifications but illustrate potential performance.

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