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Mycoplasmosis Prevention and Control in Broiler Breeders and Broilers

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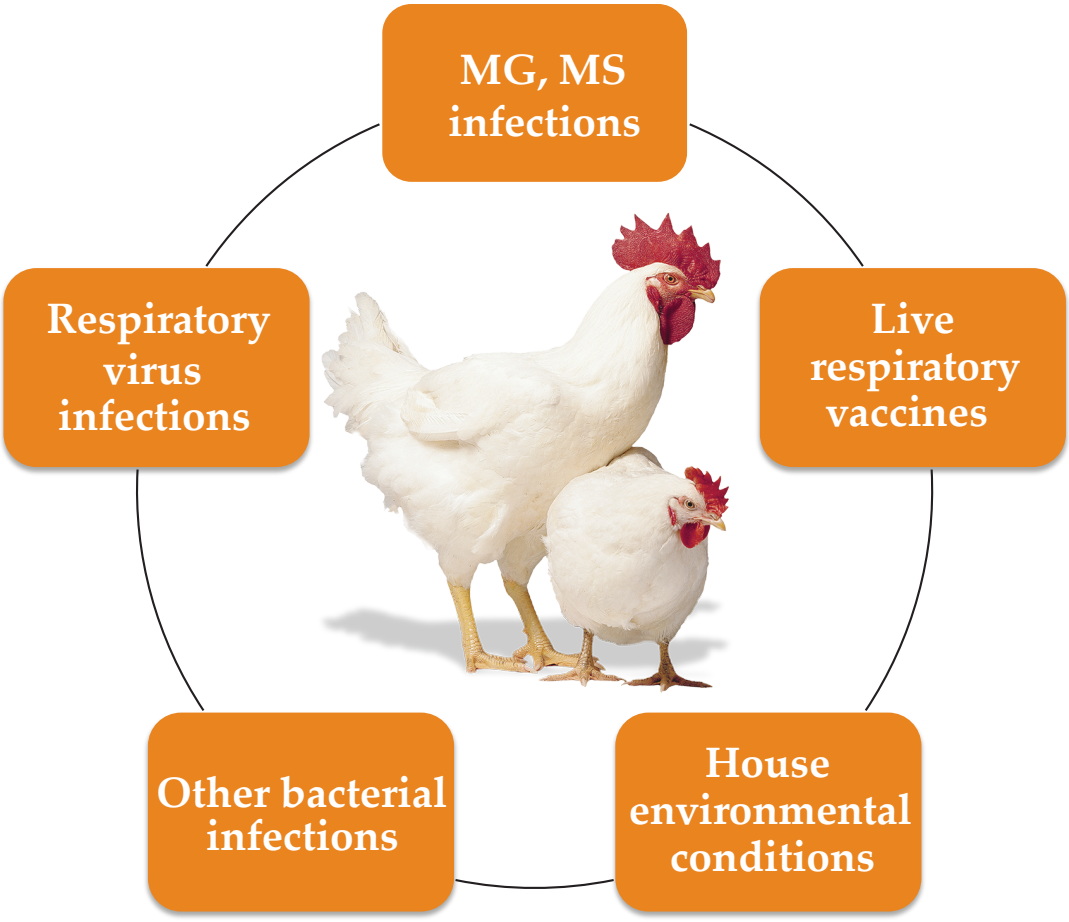
Background Information

Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) are bacteria that infect chickens and other birds, causing mild to severe clinical disease. These pathogenic organisms continue to evolve and cause economic losses for poultry producers in many regions of the world. There is a wide variation in characteristics within each mycoplasma species and between strains, including virulence and immunological responses. The severity of disease can be intensified by many factors (**Figure 1**). Complicated cases can result in chronic respiratory disease (CRD), which could have a highly detrimental impact on live flock performance and livability.

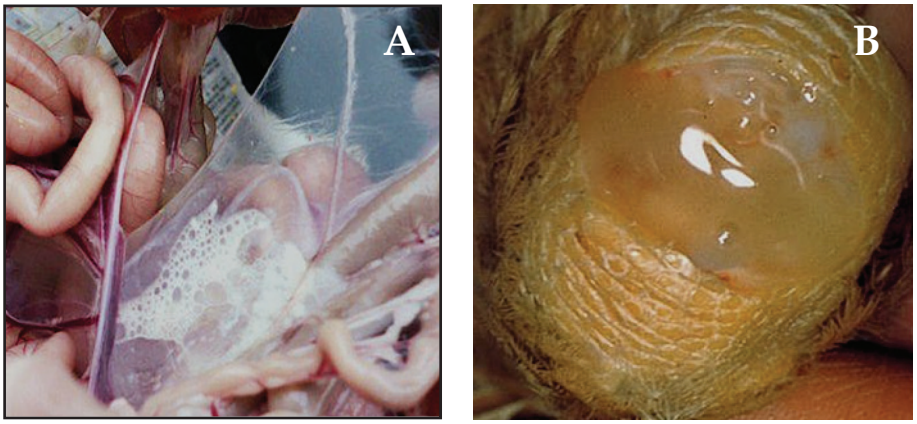
Figure 1: The severity of MG and MS clinical infections in broilers and broiler breeders can be intensified by concurrent challenges with respiratory viruses (infectious bronchitis, Newcastle disease, avian pneumovirus, avian influenza), reactions to live attenuated vaccines (Newcastle disease, infectious bronchitis), secondary infections by other bacteria (Ornithobacterium rhinotracheale, E. coli, Pasteurella spp.) and unfavorable environmental conditions (cold temperatures, wet litter, dust, high ammonia concentration).



MG infection in broilers can result in rales (respiratory noises heard in close proximity to the birds or during physical examination), coughing, nasal discharge, conjunctivitis, air-sac exudates (airsacculitis), and reduced feed efficiency. In laying hens and broiler breeders, it can also cause production drops and significant losses associated with reduced egg numbers. Embryo mortality (dead-in-shell) with air-sac lesions is also common.

Pathogenic MS strains in broiler breeders are associated with production drops and decreased hatchability. The most common signs in broiler progeny infected with MS are lameness with swollen hock joints (synovitis), respiratory disease in combination with other factors, increased condemnations (due to airsacculitis, septicemia-toxemia, peritonitis, swollen hock joints with exudate, and stunting, see **Figure 2**) and decreased performance. MS can spread faster, is more persistent, and is more difficult to manage than MG, which traditionally has been considered the more virulent mycoplasma of poultry. In adult breeders, MS problems typically start at the peak of egg production or soon after (presumably due to physiological stress). Broilers are infected vertically (from hen to embryo) and horizontally (from bird to bird), with clinical signs appearing at about 3 weeks of age. Problems with MS are more likely to occur on multiple-age broiler farms.

Figure 2: Lesions caused by MS in broiler chickens: A) Airsacculitis, and B) Swollen hock joint containing viscous, yellow-gray exudate.



Source: *Gross Pathology of Avian Diseases. T.Abdul-Azis and H.J. Barnes. AAAP. 2018*

Despite growing knowledge on the epidemiology of mycoplasmas and improvements in control strategies, costly outbreaks continue to occur, and new effects are being identified. Emerging strains of MS have caused production drops along with egg shell quality problems in both commercial egg layers and broiler breeders. Shell quality issues are characterized by egg shell apex abnormalities (EAA) or "top coning" resulting from a rough and thin shell surface with increased translucency close to the apex (**Figure 3**). These abnormalities lead to increases in cracked and broken eggs. EAA's are known to occur predominantly in MS positive hens with concurrent infectious bronchitis virus (IBV) followed by egg production drops.

Figure 3: Egg shell abnormalities (EAA) or "top coning" caused by MS infection in hens.

Source: *Dr. Nick Dorko, Global Head of Veterinary Technical Services, Aviagen, Inc.*

With the growth of poultry production, there has been a gradual increase of bird numbers in relatively small areas leading to increased risk of exposure to pathogenic mycoplasmas. In some areas, poultry operations are situated so close to each other that from an epidemiological point of view it resembles a very large multi-age farm.

MS infections are frequent in multi-age commercial egg-laying farms in some areas, which often become reservoirs for neighboring broiler breeder flocks. Often, MS can cause silent infections that may result in seroconversion (test positive by serological assays) in broiler breeder flocks with no disease signs or apparent impact on performance. However, during the last ten years, outbreaks have caused production drops with increases in mortality followed by severe respiratory disease in the broiler progeny, increased condemnations, reduced body-weight gains, and reduced feed conversion ratios.

Possible reasons for the re-emergence of MS in broiler breeders include:

- The existence and spread of increasingly pathogenic strains in areas with a high density of farms and biosecurity challenges.
- A growing number of companies have started reduced or antibiotic-free (ABF) production systems to meet market and regulatory demands.
- The introduction of live MG vaccines along with a decreased antibiotic use.
- Improved biosecurity and increased monitoring for MG and MS combined may be resulting in better detection of MS positive flocks.
- Proximity to infected laying farms (often without any control programs).

Diagnosis

The diagnosis of mycoplasma infections involves a combination of observation and identification of clinical signs and lesions, along with serological assays and confirmatory tests. Serology is the primary method for flock screening and commonly done by analyzing sera antibodies against MG or MS by the serum plate agglutination test (SPA) or enzyme-linked immunosorbent assay (ELISA). Positive reactions are then usually confirmed by performing a hemagglutination inhibition (HI) and/or Polymerase Chain Reaction (PCR) tests.

The SPA is rapid, sensitive, and inexpensive but can result in some non-specific reactions that need to be confirmed by HI and/or PCR tests. Fresh, good quality, and unfrozen serum is required. Poor-quality serum (due to contamination or hemolysis) is a frequent cause of false-positive results. Also, serum from day-old birds or serum drawn within 3 weeks after flocks have been vaccinated with inactivated vaccines (particularly bacterins) can result in false-positive results.

ELISA testing for MG and MS is the most popular screening procedure as commercial kits are readily available, and the procedure can be automated for large scale testing. Combined MG/MS are available, providing a convenient alternative for flock screening. When using MG/MS kits, positive results must be confirmed by separate MG or MS kits and other diagnostic methods. In general, ELISA tests are more specific than SPA and more sensitive than HI tests. The HI test is less sensitive but more specific than the SPA or ELISA tests. However, HI is a complicated and time-consuming procedure generally performed in research and reference diagnostic laboratories. In addition, it takes 3-4 weeks to get positive results.

Isolation and identification of MG and MS through culture and followed by immunofluorescence testing are considered the gold standard for diagnosis. However, these are highly specialized tests that are conducted only in specialized laboratories. Furthermore, a positive diagnosis could take up to 4 weeks.

PCR testing for MG or MS has become a rapid, sensitive, commercially available alternative to isolation to confirm a presumptive serological diagnosis or to perform routine screening of flocks (i.e., alternating ELISA and PCR testing when screening is performed every two weeks). PCR testing is also useful to test birds prior to their transfer to other farms. Swabs from trachea or choanal (palatine) cleft are often used. Impressions of upper tracheal tissue can be placed on FTA cards for PCR testing and shipped to specialized laboratories. DNA sequencing can be performed on PCR positive samples to differentiate wild field strains from MG or MS vaccine strains.

Serological methods and rapid detection by PCR, described in **Table 1**, have greatly facilitated the regular screening and diagnosis of mycoplasma infections. Although in most situations, these tests work very well, it is not uncommon to have false positive or false negative results. Therefore, it is recommended to obtain at least two positive tests by two different methods to confirm a diagnosis (i.e., SPA and HI, or ELISA and PCR). Diagnostic procedures must be performed under appropriate laboratory conditions by regularly trained personnel. Personnel should follow standardized procedures (including reference positive and negative controls for each test) and use reliable reagents to ensure the quality and accuracy of the diagnosis.

Table 1: Tests for MG and MS and their uses for diagnostic purposes.***

Diagnostic Test	Screening	Confirmation	Identify Strain
Serum plate agglutination (SPA)†	Yes	Yes*	No
ELISA	Yes	Yes*	No
PCR and RT-PCR‡	Yes	Yes	Yes, followed by sequencing
HI∞	No	Yes**	No
Isolation	No	Yes	Yes, followed by sequencing

**When a second set of samples (collected 4-7 days after the initial sampling) results in a significant increase in the number of strong positives (reactors).*
***Titer ≥ 1:80*
****All tests could give some false positive results. Must have positive and negative controls for all tests. Good protocols and training are required.*
†Unfrozen sera
‡Preferred test to screen source flocks prior to male replacement.
∞Hemagglutination-inhibition

Prevention and Control

Control measures for both MG and MS are based on preventing the infection, both via vertical and horizontal transmission. Generally, infections occur via aerosols, contact with infected birds, and mechanical transmission by humans, equipment, vehicles, and litter. Distance is the best protection against aerosol infection. MS appears to be able to transfer between flocks over greater distances than MG (due to aerosols, traffic between farms and contaminated equipment, farms tools, clothing, etc.). Domestic and wild birds, including turkeys, guinea fowl, peafowl, partridges, pheasants, quail, ducks, and geese pose a significant risk of MG to breeder and broiler farms.

MG causes conjunctivitis and sinusitis in house finches and similar species in North America. MS is more commonly associated with infections in commercial layers, show birds, back-yard chickens, and commercial turkey flocks; however, other species could be susceptible. Mechanical transmission is possible; humans can carry avian mycoplasmas in their noses and on hair for up to three days. Showering-in and breaks of at least 48 hours after visiting positive flocks can help avoid mechanical transmission. Management practices such as male replacement and flock thinning can spread mycoplasmas within an operation. Precautions and regular testing must be undertaken to reduce the risk of these practices.

Based on the above, it is essential to establish and implement strict biosecurity programs, all-in and all-out production systems and prevent direct and indirect contact between clean farms and infected commercial layers, free-range flocks, back yard chickens and wild birds. All breeder and broiler farms must be wild bird proof. Further details can be found in the technical bulletins "***Best Practice: Biosecurity in the Broiler House***," and "***Best Practice: Biosecurity in the Breeder House***." These are available in Aviagen's technical library at **Aviagen.com**.

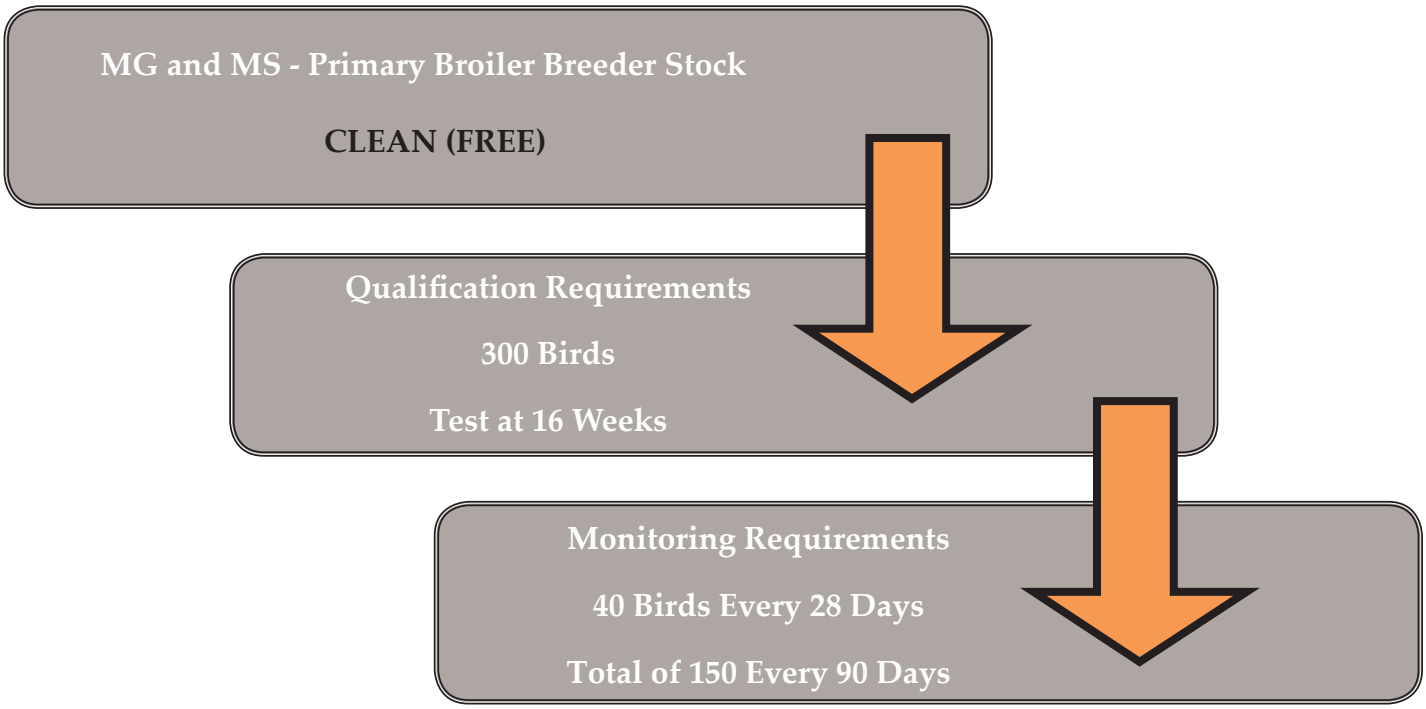
There are three methods for the control of mycoplasmas which have and continue to be used in different broiler producing areas of the world. These include:
1. Depletion (elimination of positive breeder flocks).
2. Vaccination.
3. Antibiotic intervention.

Each of these methods and their advantages and disadvantages are discussed in the following sections and summarized in **Table 2**.

Depletion

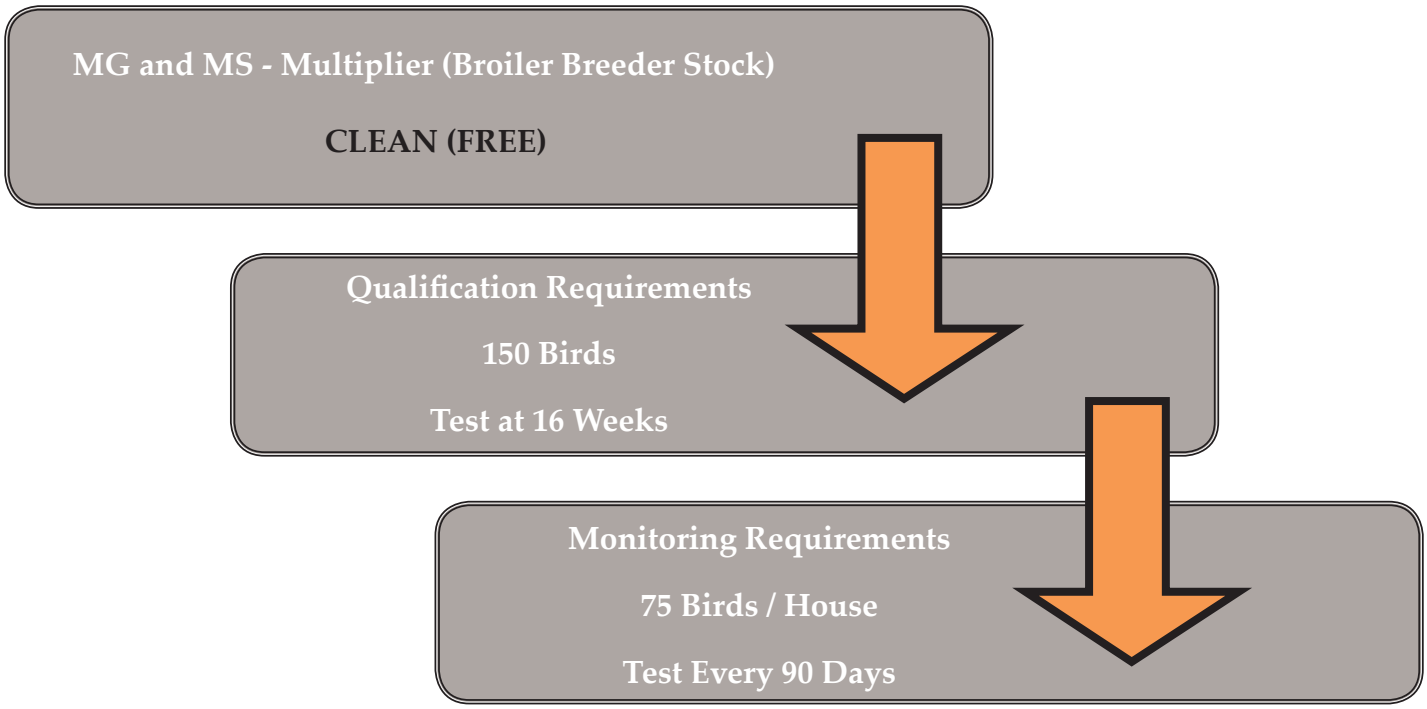
Depletion (elimination of infected breeder flocks) is the best long-term prevention strategy, and producers depend on the availability of MG and MS free breeding stock. Compliance with mycoplasma-free official certification and export requirements is mandatory for leading suppliers of day-old breeders. Suppliers of breeding stock rely on surveillance designed for prompt detection and immediate depletion of confirmed positive flocks along with their hatching eggs (if the infection occurs during the production phase). Similarly, broiler companies are establishing, managing, and maintaining mycoplasma-free status breeders through stringent biosecurity programs and regular monitoring using serology and PCR testing methods. The growing demand for broiler meat with reduced or no antibiotics makes it essential to place mycoplasma free broiler chicks and to implement effective biosecurity practices in all farms. Testing/screening procedures are required for exports and trade of day-old breeding stock and hatching eggs in many countries around the world. Many countries have monitoring programs. For example, the USDA/National Poultry Improvement Plan (NPIP) provides standardized diagnostic procedures and minimum frequency testing schedules for primary broiler breeder (GGP and GP flocks) and broiler breeder (multiplier or parent stock) flocks. Compliance with these programs requires breeding stock to be tested (using approved diagnostic procedures) to qualify and then be certified as "MG or MS Clean" (free of infection) or "MG or MS Mycoplasma Monitored." Testing scheme examples for qualifying and maintaining certification for Clean or Monitored status classifications are described in **Figures 4**, **5**, and **6**. The testing scheme examples listed were sourced from the USDA/National Poultry Improvement Program (*http://www.poultryimprovement.org/statesContent.cfm*).

Figure 4: Testing scheme example for primary broiler breeders required for certification as MG and MS CLEAN (FREE).



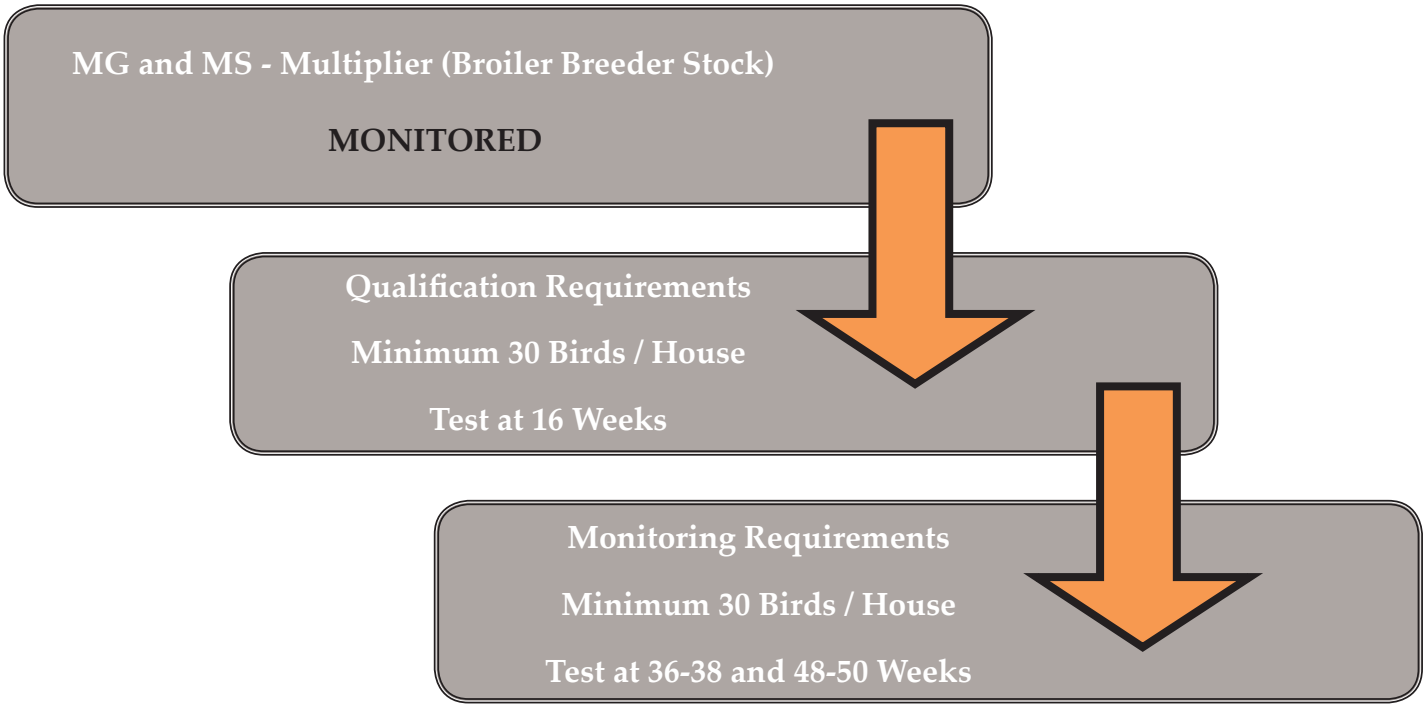
- For example, Aviagen tests GP and GGP flocks every 2 weeks during production.
- Samples are taken from 60 birds/GP and 300 birds/GGP house.

Figure 5: Testing scheme example for multipliers (broiler parent stock) required for certification as MG and MS CLEAN (FREE).



- Birds must be sourced from a CLEAN (FREE) breeder source.
- Keep birds and eggs separate from other products.
- Test minimum 30 replacement males, from male source flock, 7 to 10 days prior to move.

Figure 6: Testing scheme example for multipliers (broiler parent stock) required for certification as MG and MS MONITORED.



- Samples taken from different locations within the house.
- Sample a representative number of males and females.

Vaccination

Live MG vaccines (F, 6/85 and TS-11 strains) are commonly used in commercial layers. They are used less frequently in broiler breeders due to safety concerns, including the risk of transmission to unvaccinated flocks. In many countries, live MG vaccines are not used in broiler breeders due to potential reversion to more virulent forms, residual pathogenicity and potential for vertical transmission and respiratory disease in broilers.

A pox vectored MG vaccine can be administered via wing web during the rearing period. However, there is limited information on its efficacy, and some studies have raised questions about its ability to induce protective immunity against field challenge.

A live MS (MS-H strain) vaccine is used in broiler breeders in areas where there is an imminent risk of exposure and infection due to proximity to positive flocks, and where elimination of infected flocks is not feasible. The MS-H strain is a frozen vaccine that is administered by eye drop (required for best protection), is nonpathogenic for broiler breeders, induces persistent mucosal immunity, has limited horizontal transmission, has no risk of reversion, and confers protection against clinical signs (respiratory, egg shell and synovitis) and transmission. A few operations have used MS vaccine in conjunction with MG vaccines. Although there is no correlation between antibody titers and protection against an MS challenge, serological methods are still used to determine response to vaccination. Unfortunately, serological responses cannot differentiate vaccination from field infections, which can only be achieved using PCR diagnostics.

Vaccination with MS-H is performed during the growing period (in serologically negative flocks) and at least one month before expected field challenges (usually between 5 weeks of age and 5 weeks prior to the onset of lay). Typically, the serological response against the vaccine is variable and slow to develop. PCR testing and sequencing is used to differentiate MS-H strain from pathogenic field strains. The MS-H live strain is sensitive to all antibiotics used against mycoplasmas but is innately resistant to erythromycin. Proper vaccine handling and administration technique are critically important to maximize protection.

Additionally, it is important to consider the following factors to optimize the results of a vaccination program:
- Flocks to be vaccinated must be free of infection.
- Historic mycoplasma surveillance results should be used to design a vaccination program.
- Protection develops 3 weeks post-vaccination.
- No antibiotics should be administered prior to or following vaccination.

Live MS vaccination is not only a useful tool to control clinical signs and reduce the risk of transmission but also helps to reduce the need for antibiotics. Vaccination supports antibiotic stewardship initiatives and helps producers meet market and consumer expectations. Research studies and field experiences suggest live vaccines may help displace virulent-wild type mycoplasma and provide a better long-term strategy for operations that cannot afford to deplete positive flocks.

Ensuring optimal protection through live mycoplasma vaccines requires proper vaccine handling and administration. Vaccine failures are frequently attributed to improper vaccine handling and poor vaccine administration techniques. In addition, failures can occur as a result of the use of antimicrobials. All live mycoplasma vaccines are sensitive to antibiotics with anti-mycoplasma activity.

Inactivated mycoplasma vaccines used prior to the onset of egg production can induce high and uniform levels of antibodies against MG and MS (as demonstrated by serological assays) and prevent egg production drops and transmission. However, there are concerns about the inability of inactivated vaccines to stimulate mucosal immunity and their potential to reduce the persistency of live vaccines and protection against infection. Nevertheless, inactivated mycoplasma vaccines are sometimes used in combination with live vaccines and/or antibiotic treatments, adding a significant cost to a production system. Consequently, it becomes essential to carefully review the advantages, limitations and cost benefits of different or combined control strategies.

Antibiotic Intervention

Mycoplasmas are generally susceptible to tetracyclines (doxycycline, oxytetracycline and chlortetracycline) fluoroquinolones (enrofloxacin, difloxacin), tylosin, tiamulin, tilmicosin and combinations of lincomycin and spectinomycin. Since mycoplasmas do not have a cell wall like other bacteria, they are not sensitive to penicillin or other β-lactam antimicrobials (cephalosporins, monobactams and carbapenems) that inhibit cell wall biosynthesis. Ideally, antibiotic susceptibility should first be determined to maximize treatment efficacy. Susceptibility testing is a complex process and often not available. Therefore, antibiotic treatments are commonly prescribed by poultry veterinarians based on experience and proven cost benefits. Careful antibiotic use and compliance with regulations must be considered at all times to reduce the possibility of developing antibiotic resistance. If antibiotics are used for an extended period, it is recommended to rotate products to preserve their efficacy.

Although medication is a useful tool to reduce transmission and alleviate some clinical signs, it is not a long-term solution as it does not eliminate the risk of infection or possible transmission of wild type mycoplasmas to other farms. Once a flock is diagnosed positive, it must be considered infected for life and managed accordingly to reduce the risk to other farms in the production system. (See section on ***Control Measures for MS Positive Flocks*** below).

There is a variety of medication protocols used to treat mycoplasma positive breeder flocks, but a typical program includes the administration of antibiotics in the feed (i.e., chlortetracycline, one week/month) and drinking water (i.e., tylosin, 3-5 days/month). It has been beneficial for the broiler progeny (depending on the severity of infection) to administer an antibiotic in the pre-starter or starter feed and a different product in the drinking water prior to or following vaccination with live respiratory vaccines (i.e., live La Sota strain against Newcastle disease). The administration and dosing of antibiotics must follow the manufacturer's recommendations and mandatory withdrawal times.

Control Measures for MS Positive Flocks

In general, broiler breeder operations do not keep a MG positive flock due to the enormous risk of production drops, increased hen mortality (due to egg yolk peritonitis) severe respiratory disease (and secondary infections), and severe impacts on broiler health and live performance. Conversely, some operations may not eliminate MS positive breeder flocks since some infections cause no apparent clinical signs or impact in broiler performance. Nevertheless, once a broiler breeder flock is confirmed MS positive, it is critically important to prevent its spread to other farms, and therefore, implementation of the following procedures is recommended:

1. Strengthen biosecurity and limit traffic to the farm. Positive farms must be considered (labeled) as a risk and/or under quarantine. Visitation should be kept to a minimum.
2. Foot baths should be placed at the entrance of all houses and dedicated or disposable footwear should be worn at all times.
3. Ideally, shower-in and shower-out should be a regular practice. In the absence of showers, disposable clothing, headwear and shoe covers should be worn by all personnel at all times.
4. Feed deliveries, egg pick-ups, and visits by service personnel to the infected premises must be scheduled last during the week. A 48-hour break (including showering and change of clothes) is required before going to another farm.
5. All vehicles should be disinfected before going to another farm.
6. Egg handling and transport equipment (trays, trolleys, etc.) must be used only on the infected farm or labeled accordingly to guarantee proper cleaning and disinfection.
7. Hatching eggs from positive flocks should be separated and set only once per week or no more than twice per week. Eggs should be kept in the same incubators and hatchers and not mixed with eggs from negative flocks.
8. If eggs from negative flocks are mixed in the same incubators/hatchers as eggs from positive flocks, offspring from these eggs must be considered positive.
9. Broilers from positive flocks should be placed together and not mixed with offspring from negative flocks. Ideally, broiler progeny from positive flocks should not be placed near pullet or breeder farms.
10. Positive breeder flocks should be removed from the production system (sent to a processing plant) as early as possible (typically infected flocks are depopulated soon after they reach 50 weeks of age).
11. Where applicable, positive breeder flocks may be treated with antibiotics to reduce shedding prior to removal and transportation. Antibiotic withdrawal times must be considered before processing.
12. Movement of positive breeders going to a processing plant must be carefully planned to avoid the spread of the infection to other farms.

13. Extreme caution must be taken with the litter. Effective pest control (flies, beetles and rodents) must be implemented before the litter is removed from the infected premises. While the litter is still in the house, spray disinfection (litter, ceiling, walls and equipment) can be carried out, with curtains raised and doors closed to increase the temperature as much as possible (ideally 37-38°C or 97-100°F) for one week. Heat and drying kills mycoplasmas.
14. Positive houses should go through thorough cleaning and disinfection (C&D) procedures before repopulation. Once the birds are removed there is no host for the Mycoplasma so good C&D with the removal of the litter is always successful in eliminating mycoplasma from a farm and preventing carry-over to the next flock. Obviously, this does not guarantee against re-infection from other infected farms.

Cleaning and Disinfection

Mycoplasmas are eliminated by routine cleaning and disinfection procedures. Site cleaning must minimize the number of residual mycoplasmas. A period of downtime between flocks is crucial. More information on cleaning and disinfection can be found in the "***How To series on Cleaning and Disinfecting***." These are available in Aviagen's technical library at **Aviagen.com**.

Table 2: Mycoplasma control strategies in broiler breeders and broilers.

Strategy	Comments	Advantages	Disadvantages
MG and MS Free	Acquire free stock and maintain free status with good biosecurity practices. Needs routine surveillance.	Best livability and performance results, and ideal for reduced and antibiotic free programs.	Elimination of positive flocks may not be feasible in some high density areas or multiage operations.
Live MG Vaccine	Must vaccinate flocks with negative status prior to field challenge.	Protection against clinical effects caused by field challenge. May help displace field strain.	Safety concerns and risk of transmission to unvaccinated and broiler flocks.
Live MS Vaccine	Must vaccinate flocks with negative status prior to field challenge.	Protection against clinical effects caused by field challenge. May help displace field strain.	Positive serological response cannot differentiate from field infections. Sensitive to antibiotics.
Killed Vaccines	Induce high antibody levels. No correlation between antibody titers and protection against infection.	Can help prevent production drops and transmission.	Do not induce mucosal immunity or protect against infection. Silent infections pose a risk of transmission to broilers.
Antibiotics	Positive breeders and broiler may need medication.	Alleviate clinical signs and reduce shedding and risk of transmission.	Does not prevent transmission. Risk of drug resistance. It can mask field infections.

Summary

- MG and MS are pathogenic organisms that continue to evolve and cause economic losses in broiler breeders and broilers around the world.
- Respiratory disease, alone or in combination with other complicating factors, leads to reduced broiler live performance and increased condemnations.
- MS can cause leg problems in broilers characterized by swollen hock joints and inferior egg shell quality in broiler breeders.
- Both MG and MS can spread by vertical and horizontal transmission.
- Most recently there has been a resurgence of MS due to proximity to infected commercial laying flocks, biosecurity challenges, vaccination against MG, and reductions in antibiotic use.
- Procuring MG and MS certified free breeding stock and maintaining flocks free of infection through the establishment and implementation of biosecurity and monitoring programs are the best long-term prevention strategies.
- Antibiotic-free production is greatly enhanced by having breeders and their broiler progeny free of mycoplasma infections.
- Vaccination against MG and/or MS could be a tool to prevent the adverse effects of clinical infections in the face of imminent exposure and infection.
- Vaccination may be the best next alternative for operations that cannot afford to eliminate mycoplasma infected flocks.
- The success of vaccination depends on avoiding the use of antibiotics with anti-mycoplasma activity and by proper vaccine handling and administration.
- Antibiotic treatment is the last resource alternative. Antibiotics are not a long-term solution and do not prevent infection or transmission.
- Once a breeder flock is diagnosed as mycoplasma positive, it is critically important to enhance biosecurity practices to avoid transmission to other farms.
- Routine mycoplasma monitoring/screening by ELISA and confirmatory testing by PCR methods are increasingly common to determine the health status of breeder flocks and their progeny.

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