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Invited Review—

Control of Avian Mycoplasma Infections in Commercial Poultry

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SUMMARY. Control of pathogenic avian mycoplasmas can consist of one of three general approaches: Maintaining flocks free of infection, medication, or vaccination. Maintaining flocks free of pathogenic mycoplasmas consists of maintaining replacements from mycoplasma-free sources in a single-age, all-in all-out management system. Good biosecurity and an effective monitoring system are necessary aspects of this program. Medication can be very useful in preventing clinical signs and lesions, as well as economic losses, but cannot be used to eliminate infection from a flock and is therefore not a satisfactory long-term solution. Vaccination against *Mycoplasma gallisepticum* (MG) or *M. synoviae* (MS) can be a useful long-term solution in situations where maintaining flocks free of infection is not feasible, especially on multi-age commercial egg production sites.

RESUMEN. *Comentario por Invitación*—Control de las infecciones por Micoplasma en la avicultura comercial.

El control de los micoplasmas patógenos aviares puede estar relacionado con uno de los tres siguientes enfoques: Mantenimiento de los lotes libres de la infección, utilizando la medicación, o practicando la vacunación. El mantenimiento de los lotes libres de la infección con micoplasmas patógenos consiste en mantener las aves que se reciben procedentes de parvadas libres de micoplasma, en granjas de una sóla edad practicando el sistema de manejo todos dentro todos fuera. Los aspectos necesarios de este programa consisten en mantener buena bioseguridad y un sistema efectivo de seguimiento y análisis de laboratorio. La medicación puede ser muy útil para prevenir los signos clínicos y las lesiones lo mismo que las pérdidas económicas, pero no puede usarse para eliminar la infección de un lote y no es una solución satisfactoria a largo plazo. La vacunación contra *Mycoplasma gaalisepticum* y *Mycoplasma synoviae* puede ser una solución útil a largo plazo en situaciones donde no es posible mantener los lotes de aves libre de micoplasma, especialmente en granjas de producción comercial de huevos que tienen múltiples edades.

Key words: *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis*, *Mycoplasma iowae*, control, vaccination, medication

Abbreviations: ELISA = enzyme-linked immunosorbent assay; HI = hemagglutination inhibition; IGSR = intergenic spacer region; MG = *Mycoplasma gallisepticum*; MI = *M. iowae*; MM = *M. meleagridis*; MS = *M. synoviae*; PCR = polymerase chain reaction; RAPD = random amplified polymorphic DNA; SPA = serum plate agglutination

Control of avian mycoplasma infections is, in theory, quite simple and straightforward, especially because the pathogenic avian mycoplasmas are egg transmitted and lack a cell wall, rendering them susceptible to environmental influences. One begins with mycoplasma-free replacement stock that is placed on a single-age farm with all-in, all-out type management, good biosecurity, and a consistent monitoring program. This system has been mostly successful in the United States, and many other parts of the world, but the goal of complete control of avian mycoplasma infections still seems to be beyond our reach. We now have geographic areas with large poultry populations of various types, commercial egg production has gone to large multi-age complexes, and local reservoirs of infection have not been well identified or controlled. Multi-age commercial egg complexes are mostly positive for *M. gallisepticum* (MG) and *M. synoviae* (MS), and in some parts of the world, both infections are widespread in commercial broiler production. Nevertheless, commercial broiler and turkey production in the U.S., and much of the rest of the world, is free of pathogenic mycoplasmas.

Control of poultry mycoplasmas consists of three general aspects: prevention, medication, or vaccination. In this discussion, I will begin with a general summary of the biology of the mycoplasmas, followed by discussions on control by eradication, medication, and vaccination.

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Biology of the mycoplasmas. Mycoplasmas are small prokaryotes that lack a cell wall and are bound by a plasma membrane (115). Members of the genus *Mycoplasma* are members of the class Mollicutes (division Tenericutes) and are characterized by 16S rRNA gene sequence, differentiation from other species by serologic tests, and by a description of phenotypic characteristics (18). Mycoplasmas are characterized by a small genome (580–1350 kb), low G+C ratio, a requirement for cholesterol for growth, optimum growth at 37 C, and animal and human hosts (115). For diagnostic purposes, isolates are identified by immunofluorescence of colonies on agar (128) or by species-specific polymerase chain reaction (PCR); or for isolates of other or unknown species by sequencing of the 16S rRNA gene (47,136) or the 16S–23S intergenic spacer region (IGSR) (59). The complete genomic sequences of several *Mycoplasma* species, including MG (112) and MS (134) have been published.

Mycoplasmas require a protein-rich growth medium containing serum, along with penicillin and thallium acetate to inhibit contaminants. They are relatively slow growing. Procedures for the cultivation and detection of avian mycoplasmas are available (76). Mycoplasmas tend to be host-specific, but some species may have the ability to colonize several host species (75). Relatively little is known about the pathogenesis of mycoplasma infections, but it is clear that there is a requirement for attachment to host cell surfaces (7,92,111). Most mycoplasmas are relatively noninvasive but some species, including MG, are known to have the ability to penetrate cells (140). Phenotypic variation of expression of major immunogenic surface proteins may also be a major factor in pathogenesis (51,62,111,148).

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There are more than 120 named *Mycoplasma* species listed on the National Center for Biotechnology Web site (http://www.ncbi.nlm.nih.gov/), and more than 20 species are known to infect avian hosts (76). Of these, MG and MS are the major pathogens, and *M. meleagridis* (MM), and *M. iowae* (MI) are of importance in turkeys. *M. imitans*, a species closely related to MG, has been shown to produce respiratory disease in red-legged partridges (*Alectoris rufa*) and a mild respiratory disease in chickens and turkeys (42,43,44); *M. imitans* has not been identified in North America. The most commonly isolated commensal poultry mycoplasmas are *M. gallinarum* and *M. gallinaceum*. Both are commonly found in multi-age commercial layers and back-yard flocks and are important because they grow very rapidly and tend to overgrow the pathogenic species in *in vitro* culture (76).

Control by prevention. Because all mycoplasmas pathogenic for poultry are vertically transmitted, obtaining replacement stock from mycoplasma-free sources is essential. The major poultry breeders have eradicated MG, MS, and MM from their genetic lines, and significant progress is being made to eliminate MI from genetic lines. There is little published information on the eradication of MG and MS from genetic lines, but it most likely involved elimination or reduction of egg transmission by egg dipping (9,22,56,104) or egg heating (144), followed by rearing small groups of progeny and then eliminating those groups found to be infected. Elimination of MM was accomplished by a combination of egg dipping and injection of individual eggs with antibiotic (50,95). Egg injection is most likely being used as an aid to elimination of MI infection. In the event of infection of genetic lines or grandparent flocks with MG, MS, or MM, the major breeding companies will eliminate infected flocks.

An effective biosecurity program should be adequate for maintaining flocks free of mycoplasma infection. However, there are now large concentrations of poultry in small geographic areas, thereby increasing the probability of exposure, especially when there are lapses in biosecurity (74). As has been shown with *M. hyopneumoniae* infection in swine (88), it is assumed that the greatest risk factor for avian mycoplasmas is the presence of infected flocks nearby. We also know now that MG and MS are capable of surviving in the environment for longer periods than previously assumed, thus increasing the risk of contaminating flocks by indirect exposure (24,123,125). It is likely that formation of biofilms may be important in the environmental survival of mycoplasmas (94).

A consistently applied monitoring system is essential for prevention of mycoplasma infections. In the event of an outbreak in a flock, early detection is of utmost importance in order to prevent contamination of other flocks. Adding young males to improve fertility in older breeder flocks is especially hazardous. In the United States, the National Poultry Improvement Plan (4) oversees monitoring and control of MG, MS, and MM in breeding flocks. Serologic testing of 300 samples prior to the onset of egg production, and monitoring every 60–90 days, is an effective program. In general, monitoring fewer samples more frequently is preferred to testing large numbers of samples less frequently.

Sera are screened using the serum plate agglutination (SPA) test (2,99,105) or ELISA (34), and reactors are confirmed by hemagglutination-inhibition (HI) (26,36,133). The SPA test is simple, cheap, and sensitive, which makes it ideal for a screening test; however, nonspecific reactors are common, especially within a few weeks after inoculation of oil emulsion vaccines against other disease agents (11,13,53,118). The HI test is commonly used to confirm agglutination or ELISA reactors. Unfortunately, there are no serologic tests available for the detection of MI antibodies.

Isolation and identification of the organism is the "gold standard" for diagnosis of mycoplasma infections. Procedures for isolation and identification, including formulations of commonly used media, are available (76,80). However, mycoplasmas are slow growing and are commonly overgrown by commensals such as *M. gallinarum* and *M. gallinaceum*.

The polymerase chain reaction test is rapid, sensitive, and specific, and is often used instead of culture to detect the presence of specific mycoplasmal DNA. PCR procedures are available for MG or MS (46,60,70,93,101,114). Real-time PCR procedures for MG have also been described (19,20,96). PCR procedures for MM (12,45,98,151) and MI (12,45,81,152) have also been published. Because there is no serologic test for MI, diagnosis depends on culture or PCR from dead-in-shell turkey embryos; from joints or esophagus of young poults showing signs of stunting or synovitis; or from the reproductive tract of adult turkeys (124).

Medication. Since mycoplasmas lack a cell wall, they are resistant to β-lactamic antibiotics such as penicillins or cephalosporins. However, they tend to be sensitive to macrolides, tetracyclines, fluoroquinolones, and others. Procedures for the *in vitro* sensitivity testing of mycoplasmas have been published by Hannan (58), and there are several reports of *in vitro* sensitivity determinations (14,77,82,135). There are also numerous reports of efficacy testing of various antibiotics in infection models (23,52,57,67,68,106,130,135) and in naturally infected birds (126). Antibiotic resistance has been reported (14,48,97,109, 141,150). Antibiotic medication has been used to reduce egg transmission (3,30,107,145) and to improve egg production in MG-infected commercial layers (108). Dipping of hatching eggs in antibiotic solution or injection of individual eggs has been used to reduce or eliminate egg transmission of MG and MM (9,22,50,56,95,121).

Currently tylosin or tetracyclines are the most commonly used products in the United States for reduction of egg transmission or for prophylactic treatment to prevent respiratory disease in broilers or commercial turkeys. Highly effective products, such as enrofloxacin or tilmicosin, are not approved for use in poultry in the United States. A typical treatment program in infected breeding stock may consist of continuous medication in the feed or treatment for 5–7 days each month. Treatment may reduce the populations of MG in the respiratory tract (28), thus potentially reducing the risk of spread to neighboring flocks. Medication of naturally infected birds with enrofloxacin was highly effective in reducing or eliminating upper respiratory infection with MG, but had little effect on populations of MS (126). There is one report of apparent eradication of MS from a flock after intensive treatment for control of *E. coli* (41).

Nevertheless, even though antibiotic medication can be an effective tool for the reduction of egg transmission, clinical signs, and lesions, medication cannot be depended upon to completely eliminate infection from a flock (116,126), and continuous use may result in the development of resistance. Antibiotic medication can be effective and useful in preventing economic losses associated with avian mycoplasma infections, but it should not be considered to be a long-term solution.

Vaccination. In situations where maintaining flocks free of MG or MS infection is not feasible, usually in multi-age commercial layer operations, vaccination can be a viable option. Choices include inactivated, oil-emulsion bacterins, live vaccines, or a recombinant live poxvirus vaccine containing and expressing key protective MG antigens.

There are reports of the use of MG bacterins in chickens to protect against respiratory signs, airsacculitis, egg production losses,

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and reducing egg transmission (54,55,61,69,117,122,142,146,147), while other reports cited a lack of detectable efficacy (71,110). Bacterin-vaccinated chickens were marginally more resistant to intratracheal challenge infection with low doses (129) and harbored somewhat lower populations of MG in the upper respiratory tract (73). Bacterin-vaccinated chickens had minimal resistance against contact infection in a contact-challenge model (39). There have been reports of local inflammatory reactions at the injection site (29). Other adjuvants, such as liposomes (6) and iscoms (127), have been evaluated.

The major advantage of oil-emulsion bacterins is that protection against economic losses can be obtained without the introduction of a live-vaccine strain. Disadvantages are cost, the requirement for handling individual birds, and the relative lack of protection against colonization of field challenge strains of MG.

There are three live MG vaccine strains licensed in the United States: F strain, marketed in the United States by Schering-Plough Animal Health (F Vax-MG®) and Lohmann Animal Health (*Mycoplasma gallisepticum* vaccine®); 6/85 (Mycovac-L®), marketed by Intervet; and ts-11 (MG ts-11®), marketed in the United States by Merial and Internationally by Bioproperties, Ltd. (With the recent merger of Schering-Plough and Intervet, the Schering-Plough F strain vaccine will now be marketed in the United States by Fort Dodge Animal Health, but Schering-Plough will continue to market it internationally). Whithear (137) provides an excellent review of the use of live MG vaccines.

The original F strain was first described by Yamamoto and Adler (143) as a typical virulent MG strain, but it was later described as having moderate virulence (120). It was subsequently used as a live vaccine for the vaccination of commercial layers (21,35,87). There have been numerous laboratory challenge studies showing the efficacy of F strain vaccination of chickens in prevention of airsacculitis, respiratory signs, egg production losses, and egg transmission (16,27,54,55), but it was found to be too virulent for use in broilers (119). F strain is virulent for turkeys (85) and has been shown to have been responsible for outbreaks of clinical MG infection in turkeys in the field (83). F strain was shown to be egg transmitted when chickens were challenged during production, but chickens challenged prior to the onset of production did not shed through the egg (86). In addition, F strain was shown to be relatively poorly transmissible in floor-pen trials (72), but circulated among unvaccinated flocks on a commercial egg-production complex (132). Aside from commercial egg complexes, there are no reports of inadvertent F strain infection in unvaccinated chickens. F strain vaccines are lyophilized and may be administered by spray, eye drop, or in the drinking water (102).

The 6/85 vaccine strain was developed from a U.S. field isolate, was shown to be avirulent for chickens and turkeys, induced protection against air sac lesions, and was genetically stable (32,33). It was detectable in the upper respiratory tract for 4–8 weeks after vaccination and did not induce a detectable serologic response (1,32,84). The strain was very poorly transmissible to chickens and turkeys in a floor-pen trial (84). Bird-to-bird transmission of a challenge strain was lower in vaccinated birds than in controls, but the reduction in transmissibility was judged to be inadequate for stopping the spread of wild-type strains in vaccinated birds in the field (38). Vaccination prior to the onset of lay had no effect on egg production and egg quality in unchallenged chickens (15) and did not exhibit increased virulence after backpassage in chickens or turkeys (32,149). However, virulent isolates indistinguishable from 6/85 have been isolated from turkeys with respiratory signs and lesions (79), and 6/85-like strains were isolated from vaccinated commercial layer chickens exhibiting respiratory signs, but the isolates appeared to be of low virulence in experimentally challenged chickens (131). There are no publications on the use of the 6/85 vaccine strain in turkeys, but there has been limited use in the field. The 6/85 vaccine is available as a lyophilized product, and vaccination by spray is recommended.

The ts-11 vaccine strain originated from an Australian field strain which was chemically mutagenized and selected for a clone which would grow better at 33 C than at 37 C. It was shown to be safe and efficacious for use in chickens (138,139). The ts-11 strain has also been shown to be safe in turkeys, induces a slow antibody response in chickens, is poorly transmissible from bird to bird, and demonstrates efficacy in laboratory and field situations (1,5,10,25,137). The poor systemic antibody response after vaccination is not associated with lack of protection against challenge (103). The ts-11 strain persists in the upper respiratory tract for the life of the flock and induces long-lived immunity (137). There are no deleterious effects on egg size, egg production, or egg quality in vaccinated, unchallenged chickens (17). In a field study vaccinated broiler breeders exhibited improved egg production and decreased respiratory disease in their broiler progeny (5). The ts-11 vaccine is provided as a frozen product, and administration by eye drop is recommended.

A recombinant fowl pox vaccine that contains and expresses protective MG proteins has been recently introduced by Biomune. There are no published reports concerning its efficacy or safety. However, it has the advantage of not introducing a live MG vaccine strain into a flock. Since no circulating antibodies are detected after vaccination, a serologic response would be an excellent indicator of colonization by a wild-type field strain.

Few comparative studies of MG vaccines have been conducted. In one such study of live vaccines in young chickens, ts-11 and 6/85 induced a milder post-vaccination reaction than did F strain and did not persist as long as did F strain in the upper respiratory tract. After challenge, all vaccines induced protection against airsacculitis, and F strain-vaccinated birds had the fewest and mildest post-challenge air sac lesions (1). In comparing serologic responses to vaccination, ts-11, F strain, and 6/85 all induced a slow or negative antibody response, as measured by serum plate agglutination and HI, but antibodies were detected earlier by ELISA (102). Real-time PCR reactions have been designed to quantitatively differentiate between colonization with vaccine strains and the R challenge strain in vaccination challenge studies (113). Generally, it is considered that vaccination with ts-11 or 6/85 induces a milder respiratory post-vaccination reaction and somewhat lower immunity that does F strain, but the milder strains are preferred when there is danger of inadvertent infection of susceptible neighboring flocks, especially turkeys.

An ideal MG vaccine should provide protection against colonization after exposure to wild-type strains. Chickens vaccinated with F strain had an ID_{50} 3.8 $\log_{10}$ lower than unvaccinated birds when challenged with a virulent tylosin-resistant MG strain (27). The $\log_{10}$ ID_{50} of chickens vaccinated 0, 1, or 2 times with an inactivated, oil emulsion MG bacterin was 2.9, 3.4, and 3.7, respectively (129), and bacterin-vaccinated chickens had marginally lower populations of MG in the upper respiratory tract after challenge (73). In a pen-trial study, F strain vaccine displaced the virulent R strain after contact challenge, but ts-11 or 6/85 did not (78). In a long-term vaccination study in a small, multi-age commercial layer complex, F strain displaced the resident wild-type strain, but after vaccination was terminated, F strain continued to cycle among flocks. After vaccination of all replacements with ts-11 for one production cycle, MG could no longer be detected on the complex (132).

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In using live MG vaccines, adequate vaccine titer and proper administration are essential for colonizing the respiratory tract and inducing adequate immunity (31,113,137), especially when using vaccine strains that may spread poorly from bird to bird. Vaccines should be properly handled and stored to ensure adequate titer. The ts-11 vaccine, which is presented as a frozen product, should be stored in liquid nitrogen, on dry ice, or in a −70 C freezer. As with all MG strains, it is only stable for a few weeks at −20 C.

A temperature-sensitive mutant of an Australian field strain of MS has been shown to be safe and efficacious for chickens (64,65,66,89,90,91,100). It has not been licensed for use in the U.S., but it has proven to be beneficial in several areas of the world that have problems with virulent MS infections.

When live vaccines are being used, it becomes important to be able to detect challenge with wild-type strains and to distinguish between wild-type strains and the vaccine strain. A PCR procedure using arbitrary primers (random amplified polymorphic DNA [RAPD]), has been widely used to differentiate among MG strains (37,49). RAPD is very fast and useful for distinguishing among strains, but it is not highly reproducible, so that the strains being compared must be examined simultaneously; also, a pure culture is required. More recently, PCR reactions that amplify portions of genes that show polymorphisms among MG strains, such as *mgc2* or the IGSR between the 16S and 23S rRNA genes, have been developed as diagnostic primers (40,46,114). For MS, a PCR based on the *vlhA* gene has been developed (8,63). Sequencing and alignment of such PCR products has been very useful for rapidly identification of specific strains. In addition, it has been possible to prepare databases with such sequence data.

Although vaccination for MG or MS can be a useful tool, especially on multi-age commercial egg-production sites, vaccination should be limited to situations where maintaining flocks free of infection is not feasible. Vaccines should be potent and properly administered before field exposure occurs. The type and strain of vaccine used should take into consideration various factors such as cost, route of administration, virulence of local challenge strains, and the potential for inadvertently exposing susceptible neighboring flocks.

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