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Prevention and Control of Avian Mycoplasmas

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Efforts in the United States to control *Mycoplasma gallisepticum* (MG) began in the 1960's, primarily as a response to high condemnations from airsacculitis after the initiation of USDA post mortem inspection of poultry. Somewhat later, *M. synoviae* (MS) and *M. meleagridis* (MM) were added to the program. Since then, significant progress has been made in controlling Mycoplasma infections in turkey and chicken breeding stocks. Voluntary MG control programs in the U. S. are administered under the National Poultry Improvement Plan; testing provisions and protocols are provided in their official publication (1). The majority of poultry production in the U. S. is Mycoplasma-free; however, MG and MS infection are common in commercial egg production flocks. Unfortunately, in spite of increased efforts at control, outbreaks continue to occur.

There have been changes that have resulted in an evolving situation in MG control, both in the United States and worldwide. These include changes in the poultry industry itself, improved detection methods, better understanding of the agent and its pathogenesis, and improved control methods.

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CHANGES IN THE POULTRY INDUSTRY WHICH AFFECT MYCOPLASMA CONTROL

In most modern poultry producing areas of the world, the emphasis on the control of Mycoplasma infections has been centered around maintenance of Mycoplasma-free breeding stock and keeping parent and production flocks free of infection by utilizing single-age, all-in all-out farms with good biosecurity. In many parts of the world, this has been very successful, and the majority of broiler, turkey and egg production is free of infection. In contrast, areas with less-developed poultry industries tend to have high levels of contamination with MG and MS; this poses special problems for companies attempting to institute modern production methods.

With the rapid growth of poultry production worldwide, there has been concentration of large numbers of birds into small areas, leading to increased risk of exposure to pathogenic Mycoplasmas. In some areas, poultry production is so concentrated that from an epidemiological point of view, it is almost like a very large multi-age farm. Also, general improvements in disease control have sometimes resulted in decreased efforts in biosecurity, thus enhancing the possibilities for the spread of Mycoplasma infections (16).

There has been a tendency to drift away from all-in all-out production and to concentrate production on multi-age sites. This has been especially true for commercial egg production – the majority of egg production in the U.S. is now on multi-age sites, and this trend is developing around the world. Such multi-age production sites are mostly MS-positive, and many are also MG-positive, even though grandparent and parent stocks are generally MG and MS-free.

In many locations, multi-age management of broiler breeders or broilers may occur. In turkey production, multi-stage production farms, on which 2 or even 3 different ages are maintained, are becoming quite common.

Therefore, in spite of sometimes-heroic efforts at biosecurity and improved understanding of the survival of Mycoplasmas outside the host, Mycoplasma outbreaks continue to occur.

IMPROVEMENTS IN DETECTION METHODS

The basis for control programs has centered around serological methods such as agglutination and hemagglutination-inhibition, with reactors often confirmed by isolation of the organism. More recently, commercial ELISA kits have become available (IDEXX Laboratories, Westbrook, Maine, USA; Synbiotics, San Diego, California, USA) and are becoming widely used. Such kits have excellent sensitivity and specificity, but non-specific reactions may still occur. Improvements in ELISA specificity may result from the utilization of highly purified antigens, or the use of a blocking ELISA utilizing a specific monoclonal antibody.

MG strains of low virulence typically produce a poor antibody response, and isolation from clinical specimens may be difficult (37). This may be especially true if the antigenic makeup of the MG strain involved is not a good match with the strains used to produce test antigens.

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Variability in strains and clinical responses have also been noted for MS. We have encountered situations where flocks have exhibited a low-level serological response with a low percentage of polymerase chain reactions (PCR). Such flocks have been culture negative. It has been possible to transfer such reactivity by placing SPF chickens in contact with the principals. These observations suggest that there may be atypical strains that have been undetectable with traditional diagnostic methods.

PCR represents a rapid and sensitive alternative to traditional culture methods, which require specialized media and reagents and are time consuming. At least one company (IDEXX Laboratories, Westbrook, Maine, USA) produces commercial PCR kits, which are being widely used. Although several PCR procedures have been developed, the procedure developed by Dr. Lauerman at Auburn University (23) is widely used. More recently, PCR procedures based on the *vlhA* gene for MS (15), and the *mgc2* gene for MG (14) have gained in popularity. Several different PCR methods for the detection of MG have been recently compared (10).

Such improvements in serological methods and rapid detection by PCR have done much to facilitate the rapid and accurate diagnosis of MG infection.

VARIABILITY AMONG AND WITHIN STRAINS OF *M. GALLISEPTICUM* AND *M. SYNOVIAE*

MG and MS strains are known to vary in pathogenicity and antigenicity (18, 21). Variability in pathogenicity among strains of MG has been recognized for some time (37). Significant antigenic variability among MG strains also exists (21), which could affect the sensitivity of serological tests, depending on the strain infecting the flock and the strain used to prepare antigen. There are also significant differences in virulence among strains of MS. Recently, a strain of MS was encountered in turkeys that did not induce an antibody response even though birds were culture positive in the upper respiratory tract (22). House finches (*Carpadacus mexicanus*) with conjunctivitis caused by MG has been shown to be widespread in the U.S. (25, 28). This strain has been shown to spread poorly to chickens and to be relatively avirulent in chickens (33). A house finch-like strain of MG has also been isolated from turkeys with atypically mild clinical disease (8).

Restriction Length Polymorphism (RFLP) of whole-cell DNA has been shown to be useful for differentiating MG strains (21). However, the RFLP procedure is time-consuming and laborious, making identification of specific strains a tedious procedure. More recently, Random Amplified Polymorphic DNA (RAPD) has been developed for identifying specific strains (5, 7, 11). This procedure is very simple and rapid, and has provided a routine procedure for the rapid identification of MG strains. This has proven to be very useful for epidemiological studies and for identification of specific MG strains in field outbreaks.

More recently, we have utilized a PCR for the *mgc2* (13, 14) gene of MG and the *vlhA* gene of MS (15, 32) followed by RFLP or sequencing of the PCR product to identify specific MG strains. Using this method we have been able to more closely pinpoint the identity of field and vaccine strains. Another molecular method, Amplified Fragment Length Polymorphism (AFLP), may be a more definitive way to type strains by comparing the whole genome instead of small lengths of variable genes (14).

Studies utilizing Western blots and monoclonal antibodies have shown a high degree of variability in expression of surface antigens among strains of MG; many of these proteins are variably expressed (2, 3,29). These studies have led a large effort in characterizing the variable expression of surface antigens that phase variation also occurs *in vivo*. Similar variability of surface antigen expression has now also been shown to occur among strains of MS. For example, clones of MS that are hemagglutinin negative are less virulent than clones that are hemagglutinin positive (31). The significance of such variability in the expression of surface antigens is not well understood; however, it seems logical that it would play a role in pathogenesis, serological responses, and evasion of the immune system of the host.

M. GALLISEPTICUM VACCINATION

With the advent of multi-age commercial layer complexes, control by vaccination became a consideration. The first commercially available MG vaccines were oil-emulsion bacterins (12). Bacterins protect well against airsacculitis and egg production losses, but provide less protection against colonization by field strains of MG as compared to some of the live vaccines. Bacterins require two doses for optimal protection with a higher cost of administration.

Live MG vaccines include F strain (4, 27), which has been available for some time through several manufacturers, strain 6/85 from Intervet America, Millsboro, Delaware (6), and strain ts-11, developed and widely used in Australia and licensed in the U.S. by Merial Select, Gainesville, Georgia (36).

F strain exhibits low moderate virulence in chickens (it is virulent for turkeys), colonizes the upper respiratory tract efficiently, spreads relatively slowly from flock to flock, and offers protection against losses in egg production. It provides excellent protection against colonization by challenge strains (17), and displaces the wild-type field strains present in multi-age commercial egg operations (35). Unfortunately, F strain has been implicated in field infections in commercial turkeys (24).

Strains 6/85 and ts-11 offer some advantages over F strain. They both offer protection against challenge, but are avirulent and have very limited potential to spread from bird to bird (26), thus presenting less risk to neighboring poultry flocks. F strain has better ability to displace challenge strains in pen trial studies than does 6/85 or ts-11 (17), but field experience in a commercial layer

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operation suggests that strain ts-11 may be able to displace F strain in multi-age commercial layers. After ts-11 vaccination was discontinued, the flock has remained MG-free (35).

One major concern about live MG vaccines is safety. There have been numerous instances of clinical respiratory disease caused by "escaped" F strain vaccine in turkeys; this strain should probably not be used if there is potential danger of spread to turkeys, even though it is the most efficacious strain in chickens. We are unaware of any instance of "escaped" F strain infecting chickens. There have been several instances of isolation of 6/85-like MG strains from turkeys showing clinical disease (19). In some cases there was a history of vaccination of nearby chickens or turkeys. Recently, there has been detection of 6/85-like isolates of MG in unvaccinated commercial layers housed near 6/85-vaccinated birds (34). The ts-11 strain has been detected on at least two occasions in unvaccinated chicken flocks. In both instances there was a history of possible use of contaminated vaccination equipment and in one of the instances, subsequent spread to neighboring broiler breeders. We also know that ts-11 can spread from vaccinated spike males to unvaccinated breeder females. These experiences suggest that, even though the newer vaccines are very safe, they do have the potential for spread, and their safety should be very carefully examined before a decision is made to vaccinate.

Recently, a recombinant pox vaccine, which has genes of MG inserted into its genome (Vectormune Fowl Pox – MG®, Biomune, Lenexa, Kansas, USA), has been introduced. Early field results look promising, but it is too early to determine what role it will have in overall MG control. It has a major safety advantage – no live MG organism is introduced. Also, there is no antibody response detected by agglutination, HI, or ELISA, so seroconversion suggests field exposure. A naturally occurring, avirulent strain of MG, K5054, shows promise as a safe and efficacious vaccine for chickens and turkeys (9).

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 conducted by us, 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 detected against lesions in the upper respiratory tract. The ts-11 strain appears to have limited ability to infect turkeys.

There has been relatively little work on MS vaccines. There has been one MS bacterin licensed in the U.S., but it apparently has had little field use. A temperature sensitive MS strain has been licensed for use in Australia (30), and is widely used there. It has been licensed in Mexico and some other countries, but is not available in the U.S.

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