Mycoplasma gallisepticum transmission: Comparison of commercial F-strain vaccine versus layer complex-derived field strains in a tunnel ventilated house[1]

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ABSTRACT Two simultaneous trials were conducted using a commercially available, live, F strain *Mycoplasma gallisepticum* (FMG) vaccine (trial 1) or 2 inocula of layer complex-derived MG strains (LCD-MG; trial 2). In each of the 2 trials, 4 commercial turkeys were housed in each of 2 adjoining pens immediately adjacent to air inlets. The turkeys (8/trial) were inoculated in the right eye with either a 1× dose of FMG (trial 1) or with 0.02 mL of 1 of 2 actively growing LCD-MG inocula (4 turkeys/inoculum; trial 2). In each of the 2 trials, one pen housing 4 inoculated turkeys was maintained without the addition of other poultry, whereas 16 MG-free broilers and 4 MG-free layers were added to the other pen of 4 inoculated turkeys. Within each of the trials and at increasing intervals, either 4 layers (3 pens) or 4 turkeys (3 pens) were placed down-airstream from the inoculated pens. The distance of the first pen from the inoculated turkeys was separated by the width of one pen that was empty. Succeeding down-airstream pens were situated such that the empty distance (absence of any poultry) between pens that contained poultry doubled from one pen to the next such that the final pen that contained poultry had 4 empty pens between it and the next up-airstream pen that also contained poultry. At 106 d postinoculation, all poultry were bled, swabbed for MG from the choanal cleft, and then euthanized and necropsied. No commingled poultry in trial 1 (FMG), whether inoculated (turkeys) or commingled (layers and broilers), died during the course of the trial, and 5 of the 8 FMG-vaccinated turkeys exhibited serological but not cultural evidence of mycoplasmosis. In trial 2 (LCD-MG), 2 commingled broilers died and no inoculated turkeys exhibited either serological or cultural evidence of mycoplasmosis. In both trials, no poultry housed down-airstream from the inoculated poultry showed evidence of clinical signs of mycocplasmosis and none showed either serological or cultural evidence of mycoplasmosis.

Key words: poultry, disease, mycoplasmosis, broiler, turkey

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INTRODUCTION

In the United States, turkey and chicken hatcheries and primary and multiplier breeder operations generally have adopted the various *Mycoplasma gallisepticum* (**MG**) control programs of the National Poultry Improvement Plan (**NPIP**; National Poultry Improvement Plan and Auxiliary Provisions, 1995). Through the utilization of testing and slaughter of reactor flocks, the heat treatment of hatching eggs (Yoder, 1970), and biosecurity and biosurveillance procedures (Evans et al., 2007), the entirety of the poultry breeder industry has been cleared of MG. Because of management in both the commercial broiler and turkey sectors, wherein "all-in and all-out" rearing practices are used, these 2 sectors of the poultry industry have been able to maintain MG-free commercial flocks, with the exception of sporadic mycoplasmal outbreaks. However, poultry disease management can be varied among the 3 sectors of the industry, and this is particularly true for mycoplasmal infections. In particular, the commercial table egg sector of the poultry industry is commonly infected with both MG and *Mycoplasma synoviae* (**MS**). Indeed, mycoplasmosis within the commercial table egg sector presents a much more difficult problem to resolve. This is largely due to the fact that eradication of MG or MS, the goal of "all-in, all-out" management

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practices, is impractical considering the immense number of hens, generally greater than 1 million housed in typical layer complexes. The ages of hens in these facilities are commonly 20 to 100 wk old. Furthermore, the interconnected walkways and egg belts that transport the eggs to the in-house egg processing plant, in addition to the 20- to 30-yr production service life of the facility itself, lead to a hen population that is at risk to field or wild-type strains of MG. Because of the likely probability of MG gaining access at some point during the facility's service life and the inherent egg production and mortality losses and therapeutic costs associated with MG infection of hens in production in a modern layer facility complex, many managers make the use of live MG vaccines an integral management practice, where use of such vaccines is permitted.

To control production losses, which are estimated to be 15.7 eggs/hen over a 45-wk laying cycle in MG-infected hens compared with hens maintained free from MG infection (Carpenter et al., 1981), 4 MG vaccines have been approved for restricted use in layer chickens. One of the 4 currently commercially available live MG vaccines includes Poulvac Myco F, often referred to as FMG (Fort Dodge Animal Health, Ft. Dodge, IA) which was originally licensed by the USDA in 1988. A second vaccine followed in the early 1990s, which is MYCOVAC-L, often referred to as 6/85 (Merck Animal Health, Millsboro, DE). *Mycoplasma gallisepticum* Vaccine, often referred to as TS-11 (Merial-Select, Gainesville, GA), is a third vaccine, and finally, a fourth vaccine, AviPro MG F (Lohmann Animal Health Int., Winslow, ME) was re-released in January 2011, subsequent to acquisition of Maine Biological Laboratories by Lohmann Animal Health Int. Administration of these live MG vaccines to commercial table egg poultry is currently advocated at less than 10 wk of age (**woa**) by the manufacturers and is frequently performed at 8 woa (J. Self, Cal-Maine Foods Inc., Jackson, MS, personal communication). Each of the live MG vaccines has characteristics that can be important in optimizing both the control and eradication of field or wild MG strains. The reported characteristics that differ among the vaccine strains include protection (Abd-el-Motelib and Kleven, 1993), transmission (Ley et al., 1997), pathogenicity (Whithear et al., 1990; Evans and Hafez, 1992), production characteristics (Branton et al., 1997, 1999, 2000, 2002), and the ability to displace field or wild strains of MG (Kleven et al., 1990). The foregoing differences among the various live MG vaccines are important and necessary factors to consider when developing a plan to address an MG-infected flock, and although they are important to the table egg producer, they are just as important for the broiler and turkey sectors of the poultry industry, especially where a table egg operation is in the geographic proximity of a broiler, turkey, or breeder operation.

In studies that have tested artificially created aerosols, the MG organism was recovered in gradually decreasing amounts from the air during a 6-h period, but was shown to be capable of surviving for as long as 24 h (Beard and Anderson, 1967). Based on the foregoing, many poultry husbandry personnel, managers, and veterinarians have come to believe that the MG exhaled from infected birds can initiate infection in a naïve bird once inhaled after air transmission. Indeed, in the 1960s, the poultry industry experienced 30% mortality rates in broilers due to mycoplasmosis. Furthermore, dramatic morbidity and mortality rates within the turkey sector of the poultry industry were observed as a result of mycoplasmosis in that same era (Yoder, 1965). Even today, it is the potential transmission of live MG from layer chickens to broilers, turkeys, and breeders that continues to serve as an impediment to the permitted use of live MG vaccines in some multi-poultry-sector-dense states. Therefore, the objectives of this study were to compare transmission of uncharacterized layer complex-derived–MG strains with commercially available, live F strain MG vaccine among poultry species in tunnel-ventilated housing.

MATERIALS AND METHODS

Research Facility

Two trials were conducted simultaneously in a tunnel-ventilated research facility housing 32 pens measuring 1.52 × 2.74 m each. The arrangement of experimental treatments for the studies herein is graphically represented in Figure 1. The facility was equipped with evaporative cooling and a fan capacity of 54,000 $m^3 \cdot h^{-1}$. A commercial house controller (Evolution 3000, Hired-Hand Inc., Bremen, AL) was used to control the ventilation and cooling systems to maintain an air temperature setpoint of 25°C. Pens were enclosed using 2.4-m-tall wire mesh. Each pen was equipped with tube feeders and nipple drinkers, and feed and water were provided for ad libitum consumption. Fresh pine shavings were used for bedding material, and lighting was provided with incandescent bulbs at 20 lx with an 18L:6D photoperiod. Treatment pens were selected to double the distance between successive-exposure pens as shown in Figure 1. Air flow in the facility followed the typical pattern as expected from a tunnel-ventilated facility. Air velocity in the aisles was greater than that in the pens, at 0.69 and 0.56 $m \cdot s^{-1}$, respectively. Air velocity above the pens averaged 0.76 $m \cdot s^{-1}$. All velocities in the facility exceeded the 0.25 $m \cdot s^{-1}$ threshold for still air.

Turkeys

Forty-five turkeys at 6 wk and 6 d of age (**doa**), were obtained from a commercial source that is monitored for both MG and MS under the NPIP (National Poultry Improvement Plan and Auxiliary Provisions, 1995). The turkeys were certified MG and MS free and were transported to the experimental facility. All were tested for antibodies to MG using the serum plate aggluti-

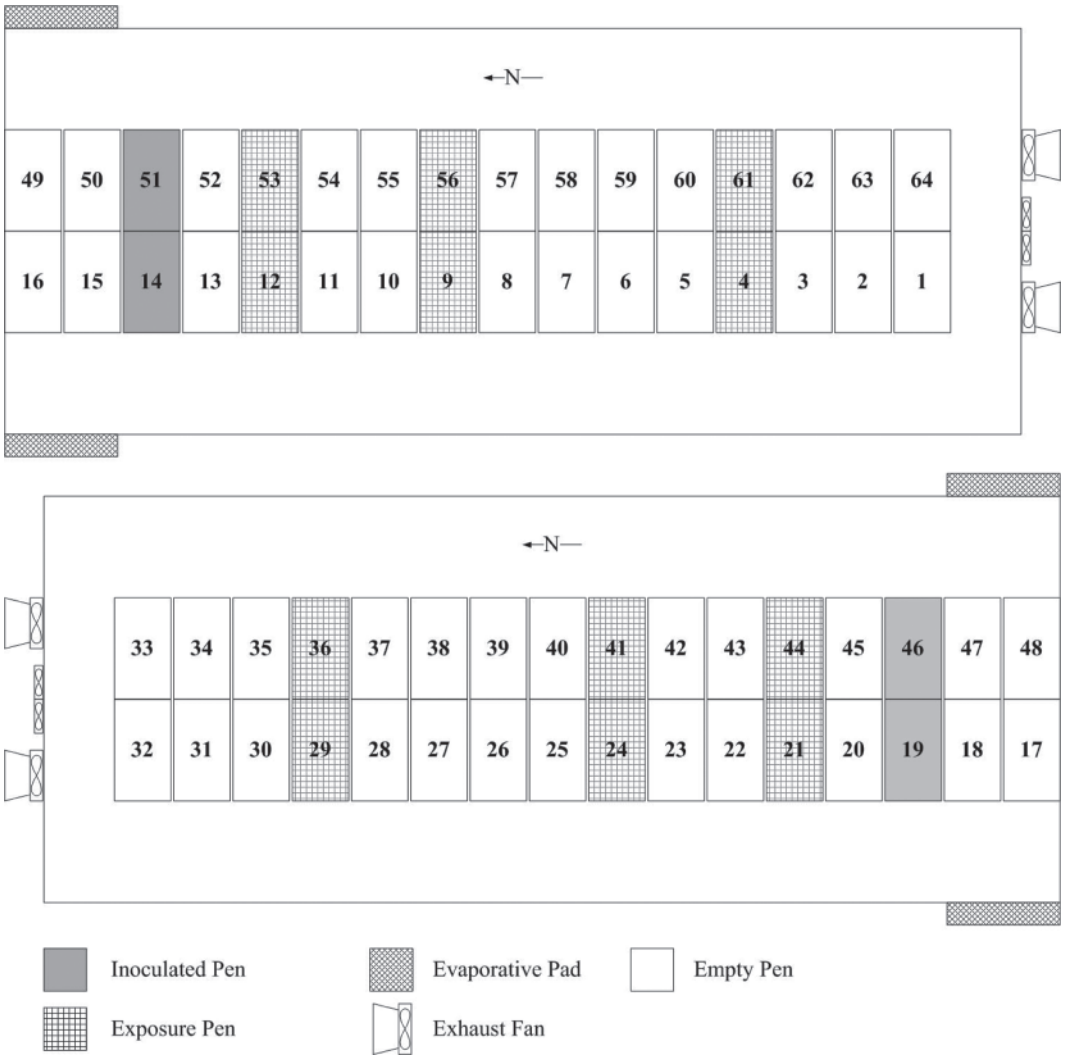

Figure 1. Plan view of pen spacing for trial 1 [commercial F strain in *Mycoplasma gallisepticum* (MG) vaccine, top] and 2 (layer-complex derived MG strains, bottom). Spacing between subsequent exposure pens is doubled for each successive pen.

nation (**SPA**) tests upon arrival. In addition, choanal cleft (palatine fissure) swabs were taken using 2.4-mm-diameter rayon-tipped swabs sterilized with ethylene dioxide as previously described (Branton and May, 1985). Swabs were immediately inoculated into sterile plastic tubes containing 2.7 mL of Frey broth medium (Papageorgiou medium; Frey et al., 1968) supplemented with an additional 35 g of thallium acetate/L and 3,000,000 IU of penicillin G/mL. Tubes were incubated at 37°C and at 90% RH until the phenol red indicator changed from red to yellow or orange. Turkeys were then placed on clean, dry, pine shavings in a 5.5 × 6.1-m section of a conventional chicken house. Feed and water were provided for ad libitum consumption. At 9 wk and 4 doa (May 4), 20 turkeys were transported to each end of the study facility and placed in 5 pens at 4 birds/pen: 2 of the 5 pens in each trial were adjacent to not only one another but also to each of the air inlets, whereas the remaining 3 pens in each trial were immediately downairstream of one of the aforementioned 2 pens. Turkeys were individually weighed, euthanized, and necropsied at termination of the study, at which time they were 24 wk and 4 doa.

Layer Chickens

Forty 1-d-old layer pullets belonging to a common genetic strain were obtained from a commercial source that is monitored for both MG and MS under the NPIP (National Poultry Improvement Plan and Auxiliary Provisions, 1995). The chicks were certified MG and MS free. Chicks were placed on clean, dry, pine shavings in a 5.5 × 6.1 m section of a conventional house. Feed and water were provided for ad libitum consumption. At 10 doa the pullets were vaccinated for infectious bursal disease via the drinking water. At 6 woa, all pullets were tested for antibodies to MG and MS using the SPA test. Choanal cleft swabs were obtained and used as described for the turkeys. At 9 wk and 4 doa (May 4), 12 pullets were transported to each end of the study facility and placed in 3 pens at 4 birds/pen: pens 53, 56, and 61 and pens 21, 24, and 29 in

trials 1 and 2, respectively. At 14 woa (June 4), an additional 4 pullets were transported to each of the 2 ends of the study facility and placed in one of the pens already housing 4 inoculated turkeys: pen 14 and pen 46 in trials 1 and 2, respectively. The layer pullets were maintained through termination of the study and then weighed, euthanized by cervical dislocation, and necropsied at 23 wk and 5 doa.

Broiler Chickens

Two hundred seventy female broiler chicks (hatch date May 5) of a single genetic strain were obtained from a commercial hatchery that is monitored for both MG and MS under the NPIP (National Poultry Improvement Plan and Auxiliary Provisions, 1995). On July 2, 16 chickens were randomly selected and placed in each of the 2 ends of the study facility within the same pens in which 4 MG inoculated turkeys had been placed 8 wk and 3 d before (May 4) and to which 4 layer pullets had also been previously added 4 wk before (June 4): pen 14 and pen 46 in trials 1 and 2, respectively. Upon termination of the present study, each broiler was individually weighed, euthanized by cervical dislocation, and necropsied.

MG Vaccination/Challenge

In both trials, treatments were administered to turkeys at 9 wk and 4 doa. In trial 1, the F strain of MG (Poulvac Myco F, Fort Dodge Animal Health, Overland Park, KS) was obtained from a commercial source. For application, the commercially available MG F strain-derived vaccine (1,000-dose vial) was suspended in sterile PBS to a final volume of 20 mL or 20 μL/1× dose. The vaccine was administered to each of the 4 turkeys housed in each of pens 14 and 51 by eyedrop inoculation at the manufacturer's recommended dose for chickens (1×). The vaccine titer was 5.2×10^8 cfu/mL. In trial 2, MG-containing inocula were derived from 2 geographically distinct multiple-age commercial egg layer complexes previously determined to be infected with multiple MG field strains (S. Kleven, University of Georgia, Athens, personal communication) termed layer complex-derived (**LCD**). Briefly, swabs were collected from the choanal cleft (palatine fissure) of 10 birds from 3 houses per farm and used to individually inoculate tubes containing 1.5 mL of Frey's (Papageorgiou) media (Frey et al., 1968) containing 3,000,000 U/L of penicillin G, 35 g/L of thallium acetate, and the color indicator phenol red. Upon indication of growth, cultures were grown on Frey's agar plates and MG was detected via the MG fluorescent antibody (**FA**) test (Baas and Jasper, 1972). To minimize laboratory passages and the possible impact of these passages on MG virulence (Power and Jordan, 1976), an inoculum (MG Cocktail A) was prepared directly from the primary (1°) broth cultures. Aliquots of 100 μL of each culture were pooled, and the resulting inoculum (MG Cocktail A) was stored at −80°C. A secondary inoculum source was derived by filtering (0.45-μm syringe-type filter, Pall Life Sciences, New York, NY) the pooled primary cultures, inoculating fresh Frey's medium, and incubating at 37°C until a color change was achieved (≈24 h). The secondary culture (MG Cocktail B) was stored at −80°C. The MG was detected in both of the MG inocula via MG-specific PCR analysis (Lauerman, 1998; Evans and Leigh, 2008). However, because of fungal contamination, MG FA-identifiable colonies were only associated with MG cocktail B, which had a titer of 8.9×10^6 cfu/mL. On the day of inoculation, inocula sources (MG cocktail A and B) were thawed on ice and applied via eye drop (20 μL/bird) to each of the 4 turkeys located in pens 19 (MG cocktail A) and 46 (MG cocktail B) in trial 2.

Diets

All diets fed to poultry were formulated to meet or exceed NRC (1994) requirements for either layers or turkeys. In pens housing turkeys alone [pens 4, 9, 12, and 51 (trial 1) and pens 19, 36, 41, and 44 (trial 2)] or in pens wherein turkeys were commingled with broilers and layers [pen 14 (trial 1) and pen 46 (trial 2)], birds were provided a nutritionally complete turkey diet, whereas pens 53, 56, and 61 (trial 1) and pens 21, 24, and 29 (trial 2) were provided the layer diets. No antibiotics at any level were added to any of the diets throughout the entirety of the study; however, a coccidiostat (Coban) was added to all the diets at 0.01% in the feed.

Feeding and Caretaking of Poultry

All of the procedures were approved by the USDA-Agricultural Research Service, Mississippi State Location Animal Care and Use Committee. During the experiments, subject handling and maintenance protocols were followed to minimize the risk of human vectoring of MG. Briefly, individual animal caretakers were assigned to individual pens. Feeding and caretaking duties were performed at the end of each workday with caretakers using footbaths upon entering and exiting the research facility. Travel between or among the pens was prohibited.

Mycoplasmal Serology

At the end of the study, each bird was bled from the left cutanea ulnae (wing) vein and tested for antibodies to the MG antigen (Charles River Laboratories International, Wilmington, MA) by SPA analysis (Yoder, 1975). Further, MG-ELISA was performed on sera collected at the end of the study trials by an independent laboratory, and interpretation of MG-ELISA results was performed according to the manufacturer's recom-

Table 1. *Mycoplasma gallisepticum* (MG) serology and culture results for poultry exposed to commercial F-strain MG vaccine (trial 1) or layer-complex derived MG (trial 2)[1]

		Turkey				Layer				Broiler			
Trial	Pen	SPA	HI	ELISA	C	SPA	HI	ELISA	C	SPA	HI	ELISA	C
Trial 1	14	2/4	2/4	2/4	0/4	0/4	0/4	0/4	0/4	2/16	0/16	0/16	2/16
	51	3/4	2/4	3/4	0/4	—	—	—	—	—	—	—	—
	12	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	53	—[2]	—	—	—	1/4	0/4	0/4	0/4	—	—	—	—
	9	0/4[3]	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	56	—	—	—	—	0/4	0/4	0/4	0/4	—	—	—	—
	4	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	61	—	—	—	—	0/4	0/4	0/4	0/4	—	—	—	—
Trial 2	46	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	1/14[4]	0/14	0/14	0/14
	19	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	44	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	21	—	—	—	—	0/4	0/4	0/4	0/4	—	—	—	—
	41	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	24	—	—	—	—	0/4	0/4	0/4	0/4	—	—	—	—
	36	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—
	29	—	—	—	—	0/4	0/4	0/4	0/4	—	—	—	—

[1]Results presented as total positive over total tested. SPA = serum plate agglutination; HI = hemagglutination inhibition; C = cultured.

[2]No poultry of this type were housed in these pens.

[3]One mortality occurred in this pen secondary to a slipped tendon at 17 wk of age, and the serology and culture results for this bird are included here.

[4]Two mortalities occurred in this pen, and only 14 broilers were tested.

mendations (IDEXX Laboratories, Westbrook, ME). In addition, all sera that tested positive with the MG-ELISA test were also tested using the hemagglutination inhibition (**HI**) test (Yoder, 1975).

Mycoplasmal Isolation and Identification

At termination of the study (August 19), all birds were swabbed from the choanal cleft as previously described. Swabs were incubated at 37°C for 30 d or until the phenol red indicator changed from red to yellow or orange. A sample from each culture was inoculated onto Frey-based (Papageorgiou medium; Frey et al., 1968) agar medium and incubated at 37°C. Colonies with morphology suggestive of *Mycoplasma* spp. were examined by an agar plate FA method (Baas and Jasper, 1972) that used direct labeling of colonies stained with a polyclonal anti-FMG antibody produced in rabbits and labeled with fluorescein isothiocynate (Kleven, 1981).

RESULTS

Trial 1

Pen 14: Commingled Poultry with 4 FMG-Inoculated Turkeys. Results for serology and culture for trial 1 are shown in Table 1. No clinical signs of mycoplasmosis and no mortality occurred over the course of the study. The 4 turkeys yielded an average weight of 13.2 kg, whereas the 16 commingled broilers yielded an average weight of 5.1 kg, and the 4 commingled layer chickens yielded an average weight of 1.4 kg. Sera from 2 of the 4 inoculated/commingled turkeys exhibited a positive MG SPA test reaction and sera from the same 2 turkeys exhibited both positive MG ELISA test and HI test results, but no MG were cultured. All sera from the 4 layer chickens were MG SPA, MG ELISA, MG HI negative as well as MG culture negative. Sera from 2 broilers tested MG SPA positive, but both sera were MG ELISA and MG HI negative; however, the MG organism was isolated from 2 other broilers whose sera were MG negative by each of the serologic tests used. At necropsy, no gross lesions suggestive of mycoplasmosis were observed for any of the poultry.

Pen 51: 4 FMG-Inoculated Turkeys. No mortality and no clinical signs occurred over the course of the study. The 4 turkeys averaged 12.6 kg. Sera from 3 of the 4 turkeys were both MG SPA and MG ELISA positive, whereas 2 of those same 4 turkeys were MG HI positive, although none were MG culture positive. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 12: 4 Turkeys. There was no mortality or clinical signs, and no turkey exhibited a positive reaction to any MG serological test or for MG culture. The 4 turkeys averaged 12.8 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 53: 4 Layers. There was no mortality, although 1 of the 4 hens exhibited a positive MG SPA test reaction. No other serological tests exhibited a positive reaction and no MG was cultured. The average weight of the 4 hens was 1.4 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 9: 4 Turkeys. One turkey died subsequent to a slipped tendon at 17 woa. Sera and culture for MG were obtained from the dead turkey and at the end of the study sera and cultures for MG were likewise obtained from the remaining 3 turkeys. No sera exhibited a positive serological reaction to any test, and no bird was MG culture positive. The average weight of

the 3 turkeys at the end of the study was 13.1 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 56: 4 Layers. No hen exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 layers was 1.4 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 4: 4 Turkeys. No turkey exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 turkeys was 13.3 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 61: 4 Layers. No hen exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 layers was 1.4 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Trial 2

Pen 46: Commingled Poultry Including 4 LCD-MG-Infected Turkeys. No mortality occurred in the turkeys over the course of the study. The 4 turkeys in the commingled pen yielded an average weight of 12.9 kg, whereas 14 of the original 16 placed commingled broilers yielded an average weight of 4.9 kg, and the 4 commingled layer chickens yielded an average weight of 1.3 kg. No sera from the 4 inoculated/commingled turkeys exhibited a positive reaction for any MG serological test, although sera from one layer and from one broiler did exhibit a positive MG SPA test reaction. All cultures from all birds were negative for MG. Subsequent to euthanasia and at necropsy, 1 of the 4 turkeys exhibited a unilateral airsacculitis, which was confined to the right thoracic air sac. Necropsy results at the termination of the study showed no other poultry exhibited gross lesions suggestive of mycoplasmosis. Two of the 16 broilers died at 12 wk, 5 doa, but no attempt was made to either collect sera or cultures for growth of MG because the 2 broilers had advanced to a significant state of decomposition when discovered by the assigned animal caretaker.

Pen 19: 4 LCD-MG-Infected Turkeys. No turkey exhibited a positive reaction for any serological test or for MG culture. Cultures from all 4 birds were negative for MG. The average weight of the 4 turkeys was 13.3 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 44: 4 Turkeys. No turkey exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 turkeys was 13.1 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 21: 4 Layers. No hen exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 layers was 1.3 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 41: 4 Turkeys. No turkey exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 turkeys was 12.9 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 24: 4 Layers. No hen exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 layers was 1.3 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 36: 4 Turkeys. No turkey exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 turkeys was 13.5 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

Pen 29: 4 Layers. No hen exhibited a positive reaction for any serological test or for MG culture. The average weight of the 4 layers was 1.3 kg. At necropsy, no gross lesions suggestive of mycoplasmosis were observed.

DISCUSSION

Results of the 2 simultaneously conducted MG trials reported herein suggest that neither the commercial FMG vaccine strain nor the LCD-MG strains were effectively transmitted beyond the respective pens containing the eyedrop-inoculated turkeys. Further, within each trial, transmission within each of the pens containing commingled poultry was inefficient over the course of the 106-d studies. Similar results with differing MG strains and turkeys have been reported wherein variation in clinical signs, antibody response, sensitivity to various serologic tests, and culture positive rates were reported to occur in turkeys 95 d postinoculation (Ley et al., 1990). Also, transmission has been reported to not always occur even after prolonged periods of contact through cohabitation of MG-infected and susceptible chickens (Olesiuk et al., 1967). Transmissibility of the F strain of MG in particular, subsequent to eyedrop inoculation, has been reported to occur readily in penmate chickens during the first 4 wk postinoculation. Furthermore, in most instances, from 6 to 27 wk postinoculation, transmission to penmates became progressively slower, whereas no transmission occurred between inoculated and noninoculated chickens separated by an aisle or empty pen of at least 6 feet (1.8 m; Kleven, 1981).

Transmission of MG is thought to be more likely to occur during the acute phase of infection when the number of MG in the respiratory tract is at its maximum (Soeripto et al., 1989). Prior research with MG has demonstrated that the peak numbers of MG in the upper respiratory tract occur about 2 wk after experimental infection and then decline thereafter (Kleven, 1985; Yagihashi and Tajima, 1986). The FMG transmission from commercial FVAX-MG 1× dose, eyedrop-vaccinated broiler chickens to unvaccinated broiler chickens, which were commingled beginning 2 wk postvaccina-

tion, has been shown to occur by 5 wk (Evans et al., 2009). For the purpose of assessing the potential for airborne transmission resulting from a maximal number of the organisms in inoculated turkeys during the initial 2 wk postinoculation in each of the 2 concurrent experimental trials, commingled layers and broilers were not added until either 4 wk (layers) or 8 wk (broilers) after inoculation of the original 8 turkeys. However, according to the experimental designs of these trials, MG-free turkeys and layers were located in pens immediately down-airstream in increasing distances from the immediately up-airstream pens of MG-inoculated turkeys. Whereas no assessment of either tracheal or choanal cleft MG numbers was made during the first 2 wk of the present trials, 3 pens, each containing 4 MG-free turkeys, and 3 pens, each containing 4 MG-free layers, were immediately down air-stream in each of the 2 simultaneously conducted studies for the entirety of the 106 d, and none of the noninoculated birds demonstrated clinical signs or serological or cultural evidence of MG over the course of the study.

Despite the fact that each of the 4 turkeys in the commingled pens of both trials were inoculated with MG, sera from only 2 of the 4 FMG-inoculated turkeys exhibited a positive MG serological test reaction. However, sera from each of the 2 were positive to each of the 3 serological tests used. It is possible that the LCD-MG strains may represent strains, which, as noted by Dingfelder et al. (1991), may differ in infectivity, virulence, transmissibility, immunogenicity, and antigenicity and may present significant obstacles to control and eradication programs in commercial turkey programs. As further noted by Dingfelder et al. (1991), turkeys infected with less virulent and less immunogenic MG strains may be especially difficult to detect and confirm, based on expected clinical signs and serologic responses with commonly used tests.

Whereas MG has historically been associated with reduced feed efficiency, it is of interest that the average weights of the turkeys, broilers, and layers in the FMG-inoculated, commingled pen (pen 14) were numerically heavier than their respective counterparts in the LCD-MG-infected commingled pen (pen 46). No statistical test was applied to the weight results because the number of birds was too few, and no pen replication for any of the treatments existed. In addition, no attempt was made to monitor or record feed consumption during the course of the study because doing so would have increased the likelihood of human vectoring of the MG organism among the pens.

Further, with the death of 2 broilers within the LCD-MG-infected, commingled pen, taken together with the aforementioned weight differences between poultry in pens 14 and 46, the argument could be made that commingled poultry within the FMG-inoculated pen not only lived but also thrived compared with poultry in the LCD-MG-infected, commingled pen. However, the same pattern did not hold for the 4 FMG-inoculated turkeys housed in pen 51 compared with their 4 LCD-MG-infected counterparts in pen 19, wherein the LCD-MG-infected turkeys were numerically heavier.

Taken together, the results of these simultaneously conducted trials suggest that the USDA-approved and licensed, commercially available, live FMG vaccine is less threatening to poultry than the wild or field strains currently established in some layer complexes. Furthermore, the fact that 5 of the 8 turkeys inoculated with the commercially available, live FMG vaccine evidenced positive reactions on at least one serologic test for MG, whereas none of the sera from the 8 turkeys inoculated with the LCD-MG inocula resulted in a positive serological test reaction, suggests that the commonly used MG test reagents may not detect some wild or field MG strains as readily as they do the commercially available F strain of MG. It has already been suggested that the use of heterologous serologic test systems contribute to low seropositive rates and to the low rates of atypical MG-infected chickens and turkeys (Dingfelder et al., 1991).

Results of the present study support the notion that the F strain of MG is no more transmissible than other endemic field strains of MG, and coupled with the ability of the F strain to displace field or wild strains of MG, these features have enabled it to be used in the eradication of MG from multi-aged flocks. Indeed, results of the present study also suggest that where F strain MG is used, the commonly used testing reagents are more capable of detecting its presence than that of the LCD-MG used in these studies. Thus, this offers a means of assessing the presence of the vaccine strain during the timeframe for the displacement of the indigenous field or wild strains, while also using the same tests to monitor for the vaccine strain once cessation of vaccination is initiated and total MG eradication begins.

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