Effects of different vaccine combinations against Mycoplasma gallisepticum on blood characteristics in commercial layer chickens

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ABSTRACT Mycoplasma gallisepticum (MG) is a major and economically significant pathogen of avian species. When administered before lay, F-strain MG (FMG) can reduce egg production during lay, but the ts-11 strain of MG (ts11MG) does not exert this effect. Two trials were conducted to determine the effects of pre-lay vaccinations of ts11MG, MG-Bacterin (MGBac), or their combination, in conjunction with an FMG challenge overlay after peak production on the blood characteristics of commercial layers. In each trial, 160 mycoplasma-free Hy-Line W-36 layers were housed in negative-pressure biological isolation units (4 units per treatment, 10 birds per unit) from 9 through 52 wk of age (woa). The following vaccination treatments were administered at 10 woa: 1) Control (no vaccinations); 2) MGBac; 3) ts11MG; and 4) ts11MG and MGBac combination (ts11MG + MGBac). At 45 woa, half of the birds were challenged with a laboratory stock of high-passage FMG. Parameters measured in both trials were whole-blood hematocrit and serum concentrations of cholesterol (SCHOL), triglycerides, calcium, and total protein (STP). An age × treatment interaction (P = 0.04) was observed for STP between 23 and 43 woa. The STP concentration in the ts11MG and ts11MG + MGBac groups was higher at 33 woa, but was lower at 43 woa, in comparison to the Control group. Also, at 38 woa, the STP of the ts11MG + MGBac group was higher than that of the MGBac group. Although use of the ts11MG vaccine alone or in combination with MGBac may influence circulating STP concentrations when administered before lay, it remains effective in protecting layers against the adverse effect of a post-peak challenge of FMG on egg production, as was observed in a previous companion study.

Key words: bacterin, blood, commercial layer, Mycoplasma gallisepticum, vaccine

INTRODUCTION

Mycoplasma gallisepticum (MG) is an important respiratory pathogen of poultry that exhibits a wide array of virulence levels (Levisohn, 1984; Soeripto et al., 1989). Common symptoms of MG infection in chickens include respiratory rales, coughing, nasal discharge, and conjunctivitis (Ley, 2003). Economic losses associated with MG infection include poor feed conversion (Patterson, 1994), and reduced egg production (EP; Carpenter et al., 1981; Mohammed et al., 1987; Stadelman, 1988) and egg size (Branton et al., 1999) in layers. On multi-age commercial layer facilities, vaccination is the only pragmatic option for the prevention of field-strain MG infections (Kleven et al., 1984; Kleven, 2008). Various types of vaccines used against avian mycoplasmosis include inactivated oil-emulsion bacterins (MGBac) and live attenuated vaccines (LAV) (Kleven, 2008). The F (FMG), ts-11 (ts11MG), and 6/85 (6/85MG) LAV strains of MG are currently available for commercial use.

Even though earlier studies have shown that the inoculation of pullets with FMG is useful in reducing EP losses associated with field-strain MG infections (Carpenter et al., 1981), it has also been demonstrated that the inoculation of pullets with FMG may lead to a subsequent delay in the onset of lay (Burnham et al., 2002a). In addition, the vaccination of layers with FMG before the onset of lay may lead to a reduction in the total number of large eggs produced during a production cycle (Branton et al., 1999). Branton et al. (1997) reported that blood packed-cell volume and plasma protein concentrations in layers were not affected by an inoculation of FMG up to 4 wk after it was administered at 60 wk of age (woa). In another study, Burnham et al. (2003b) noted that the inoculation of layers with FMG at 12 woa did not alter the concentration or physical properties of their lipoproteins, including the fractionation of cholesterol among lipoproteins. On
the other hand, Burnham et al. (2002b) suggested that the effects of an FMG inoculation at 12 woa, which resulted in a higher incidence of fatty liver hemorrhagic syndrome, ovarian follicular regression, and decreased isthmal and vaginal proportions of the reproductive tract, was the result of mutual functional disturbances in the liver, ovary, and oviduct without concomitant intestinal changes. In a companion study using the same birds, Burnham et al. (2003a) observed an increase in whole-blood hematocrit (HCT) at 20 woa in birds inoculated with FMG at 12 woa, while serum triglyceride (STRIG) and plasma protein concentrations were increased at 22 woa. They explained that the initial increase in these blood parameters is suggestive of a compensatory response in these birds to an FMG inoculation. Similarly, Peebles et al. (2007) observed higher plasma protein levels in hens at 34 woa when they had been inoculated at 22 woa rather than at 12 woa.

Nevertheless, results from the study by Burnham et al. (2003a), in which a significant decrease in the plasma protein and STRIG concentrations in FMG-inoculated birds occurred at 52 and 54 woa, respectively, in response to an FMG inoculation at 12 woa, suggests that FMG can also exert a chronic effect on the synthesis of proteins and lipids in the livers of commercial layers. In confirmation of the potential of FMG to have both relative acute and chronic effects on the blood characteristics of layers, Peebles et al. (2010) reported that the inoculation of layers with FMG at 12 or 22 woa resulted in a reduction in STRIG at 24 woa and SCA at 34 woa, but subsequently increased SCA at 58 woa.

Peebles et al. (2008) observed an increase in the plasma protein concentrations of layers at 32 woa in response to a 6/85MG inoculation at 10 woa alone or in conjunction with an FMG inoculation overlay at 22 woa. In that same study, serum calcium (SCA) concentrations were observed to increase at 47 woa when a 6/85MG vaccine was given before lay at 10 woa in conjunction with a subsequent FMG vaccine overlay at 45 woa. It was suggested in a report by Peebles et al. (2009) that a ts11MG vaccination at 10 woa alone or in combination with an FMG overlay inoculation at 22 or 45 woa may be administered without affecting HCT, plasma protein, STRIG, or serum cholesterol (SCHOOL) concentrations in commercial layers. However, it was observed in that same report that a ts11MG vaccination at 10 woa alone or in conjunction with an FMG inoculation at 22 woa resulted in an increase in SCA concentrations. This may explain the improvement in EP reported by Branton et al. (2000), when the ts11MG vaccine was used alone.

There are no data available concerning the prelay use of MGBac on the blood characteristics of commercial laying hens. Furthermore, no information has been published concerning these possible effects in response to the combinatorial prelay use of ts11MG and MGBac in conjunction with a subsequent FMG overlay vaccination administered after peak EP. Therefore, the objective of this study was to determine the effects of ts11MG and MGBac vaccines, administered individually and in combination before lay (10 woa) with and without a subsequent FMG vaccination overlay after peak EP (45 woa), on various blood characteristics of commercial laying hens.

**MATERIALS AND METHODS**

**Research Design and Treatments**

Two trials were conducted using commercially available MG vaccines that were administered in accordance with their manufacturer’s recommendations. The titers of each vaccine in each trial were reported in a companion article by Jacob et al. (2014). In each trial, the following treatments were utilized at 10 woa: treatment 1 (Control) received no vaccination; treatment 2 (MGBac/10) consisted of birds that were vaccinated with 0.5 mL of MGBac (MG-Bac, Fort Dodge Animal Health, Overland Park, KS) via i.m. injection into the left breast muscle using a 1 mL tuberculine needle and syringe; treatment 3 (ts11MG/10) consisted of birds that were eye-drop-vaccinated in the left eye with approximately 30 μL of ts11MG (Mycoplasma Gallisepticum Vaccine, Merial Select, Gainesville, GA); and in treatment 4, birds were vaccinated with both ts11MG and MGBac (ts11MG + MGBac/10) according to the previously described methods of administration for each. Each treatment group consisted of 4 replicate isolation units containing 10 birds each for a total of 40 birds per treatment group. At 45 woa, only the birds in 2 replicate pens in each of the treatment groups were overlaid with a laboratory stock of high-passage-FMG (99th passage above the unknown passage level), increasing the number of the treatments to 8. A 24-h broth culture of FMG was generated in Frey’s broth medium (Frey et al., 1968), and 20 μL of the overnight culture was applied via eye-drop inoculation. The FMG overlaid counterparts of all the above treatments were represented as Control + FMG/45; MGBac/10 + FMG/45; ts11MG/10 + FMG/45; and ts11MG + MGBac/10 + FMG/45, respectively. Handling procedures were implemented to minimize risk of cross-contamination.

**Pullet and Layer Management**

All procedures of bird handling and management were approved by the USDA-ARS Institutional Animal Care and Use Committee. One-day-old Hy-Line W-36 commercial layer chicks used in each trial were obtained from a MG- and Mycoplasma synoviae-free commercial source. The chicks were placed in a conventional house on clean, dry pine shavings with an initial flock density of 0.034 m²/bird until 9 woa. The daily artificial lighting schedule was 13 h light and 11 h dark. Further lighting details during the pullet phase were as described by Jacob et al. (2014).
In both trials, groups of 11 birds each were randomly selected at 9 woa and transferred to temperature-controlled, negative-pressure fiberglass isolation units in an environmentally controlled disease-isolation facility (Branton and Simmons, 1992), and were maintained under conditions described by Evans et al. (2012). Four birds per unit were bled, and the serum was used for the detection of any MG infections via serum plate agglutination tests at the time of placement in the isolation units. In addition, following the procedure described in detail by Jacob et al. (2014), further MG screening was performed on all birds at 9, 45, and 52 woa using choanal cleft swabs to test for the presence or absence of MG according to treatment by PCR-based DNA detection techniques (Kleven, 2008).

When the total daily EP of the birds reached 10%, one bird in each pen was randomly selected and killed to reduce the number of hens to 10 per unit (stocking density: 0.116 m²/bird) for the duration of each trial. All the biological isolation units were maintained at 23°C throughout the entire duration of the trials. Between trials 1 and 2, the location of the treatment groups within the isolation facility was rearranged to assure randomization between the trials. Beginning at 18 woa, the artificial lighting schedule was increased by 15 min/wk until a cycle of 16 h 15 min light and 7 h 45 min dark was achieved as previously described (Branton et al., 2002). Layers were maintained on that schedule through the remainder of the experiment.

**Pullet and Layer Diets**

For the duration of each trial, birds had *ad libitum* access to feed and water. In the pullet and layer phases, all birds received standard diets that met or exceeded National Research Council (1994) recommendations. The diets, as previously described by Burnham et al. (2002a), were formulated according to the age of the birds, with 5 types of diets being provided over the course of each trial as follows: starter, 0 to 6 woa; grower, 6 to 12 woa; developer, 12 to 18 woa; prelay, 18 woa to onset of lay; and layer, onset of lay to end of experiment.

**Data Collection**

Blood samples were collected from 4 tagged birds from each replicate pen at 23, 28, 33, 38, 43, 47, and 52 woa in both trials for the determination of HCT, SCHOL, STRIG, and serum total protein (STP) concentrations. In both trials, all data collected from 23 to 43 woa were designated as belonging to age interval I, and all data collected at 47 and 52 woa were designated as belonging to interval II. Data from the 2 trials were pooled and analyzed together, with trial considered as a random effect. Because blood samples were taken from the same tagged birds in their respective pens (experimental units) at multiple time periods within each interval, the data were subjected to repeated measures analysis to test for the main and interactive effects of bird age and treatment. Individual sample data within each of the replicate units were averaged before analysis. All data were analyzed using the MIXED procedure of SAS (SAS Institute, 2009). Least squares means were compared in the event of significant effects. F tests were performed to evaluate main effects and interactions, and comparison of least squares means was achieved using *t* tests (Littell et al., 2006). Effects and differences among least squares means were considered significant at *P* ≤ 0.05.

**RESULTS**

For both trials, serological screenings at 9 woa indicated that the birds had no previous exposure to MG. Furthermore, PCR assays at 9, 45, and 52 woa revealed no evidence for the presence of MG-DNA in those treatments not receiving ts11MG vaccination or the FMG.
overlayer. However, the presence of MG-DNA was detected in those that received ts11MG or FMG, and for evidence its presence did not differ between those groups that received ts11MG alone or in combination with MGBac (Jacob et al., 2014). There were significant age main effects for STRIG ($P = 0.0001$) and SCA ($P = 0.0002$) in age interval I (Table 1), and for STRIG ($P = 0.01$) in age interval II. At 23 woa, STRIG concentrations were lower than those at 28, 33, 38, and 43 woa. Also, SCA concentrations at 38 woa were higher than those at 23 and 43 woa, and those at 28 and 33 woa were higher than those at 23 woa. Mean STRIG concentrations at 47 and 52 woa (interval II) were 3,151 and 3,920 mg/dL, respectively (pooled SEM = 747 mg/dL). Mean STRIG concentrations at 52 woa were significantly higher than those at 47 woa. There was a significant age × treatment interaction ($P = 0.04$) for STP in age interval I (Table 2). The STP concentrations of the ts11MG/10 and ts11MG+MGBac/10 groups were higher at 33 woa, but were lower at 43 woa, in comparison to the Control group. At 33 and 43 woa, STP concentration in the MGBac/10 group was intermediate to and not significantly different from the other treatment groups. Furthermore, at 38 woa, STP concentrations in the ts11MG + MGBac/10 group were higher than those in the MGBac/10 group. At 38 woa, STP concentrations in the Control and ts11MG/10 groups were intermediate to and not significantly different from the ts11MG + MGBac/10 and MGBac/10 treatment groups. There was no significant treatment effect on STRIG in either age interval or on SCA in age interval I, and there was no significant age effect on STRIG in age interval II. Furthermore, there were no significant age or treatment effects for SCA or STP concentrations in age interval II, and there were no significant age or treatment effects noted for HCT or SCHOL in either age interval.

**DISCUSSION**

Similar to the current results, Peebles et al. (2008, 2009) noted that there was no layer age effect on HCT or SCHOL when blood samples were taken at 22, 24, 32, 43, 47, and 56 woa. However, it was noted in those previous studies that layer age likewise had no effect on STRIG. Therefore, upon comparison of the current results with those of Peebles et al. (2008, 2009), a significant layer age effect on STRIG was uniquely observed in the present study. More specifically, it was observed in this study that STRIG significantly increased between 23 and 28 woa. Furthermore, the current results showed only an age main effect and no effect due to vaccination treatment on SCA. This is in contrast to results reported by Peebles et al. (2009), in which the effects of ts11MG at 10 woa with and without an overlay of FMG at 45 woa was also tested. In that report, an interactive effect between age and the individual vaccination of ts11MG at 10 woa was found to occur for SCA. The SCA of birds at 22 woa was observed to be elevated in response to vaccination with ts11MG at 10 woa. Nevertheless, a subsequent FMG overlay at 45 woa, with or without a ts11MG vaccination at 10 woa, had no effect on SCA at 56 woa. However, Peebles et al. (2009) also noted, based on reports in companion studies by Vance et al. (2008a,b); that the change in SCA in response to the ts11MG vaccination was not associated with any changes in layer performance or egg quality. The lack of an effect of a ts11MG vaccination alone or in conjunction with an FMG overlay at 45 woa on SCA in this study, and the observation by Peebles et al. (2009) that the same vaccination regimens only affected SCA at 22 woa without affecting performance or egg quality, indicates that an acute SCA response is the most that may be expected after a prelay ts11MG vaccination, and that a subsequent FMG overlay at 45 woa exerts no added effect.

Peebles et al. (2009) also examined the effects of a ts11MG vaccination at 10 woa alone or in conjunction with an FMG vaccination during lay at 45 woa on various other blood parameters in addition to SCA. Similar to the results of the current study, Peebles et al. (2009) noted that for blood samples drawn at 22, 24, 32, 43,
and 56 woa, that there were no significant age × treatment interactions or treatment main effects due to a ts11MG vaccine at 10 woa on HCT, SCHOL, or STRIG between 22 and 56 woa. Peebles et al. (2009) also noted that there was no treatment main effect due to ts11MG at 10 woa alone or in conjunction with FMG at 45 woa on these same parameters at 56 woa. Both studies confirm that an individual prelay (10 woa) ts11MG vaccination, or one that is paired with a subsequent FMG overlay vaccination during lay (45 woa), has no consequential effects on HCT or on lipid (SCHOL and STRIG) levels in the blood of laying hens. Therefore, although circulating STRIG levels are known to be increased in response to pathogenic infections (Guyton and Hall, 1996), and despite the suggestion by Warriss et al. (1993) that significant increases in HCT together with increases in blood osmolality are indicative of dehydration in birds, these results suggest that the vaccination regimens employed in this study did not induce dehydration or a significant response in the blood lipid levels of these birds.

Peebles et al. (2009) also did not observe an age main effect or any vaccination treatment effect involving ts11MG at 10 woa with or without an FMG overlay at 45 woa on plasma protein concentration. The interactive effect of age with vaccination treatment on STP observed in the present results are, therefore, distinctive and in contrast to the previous results of Peebles et al. (2009). However, STP did not display a consistent response to treatment over the bird ages examined; rather, the singular increase in STP at 33 woa suggests that MG may be capable of interfering with protein metabolism in the liver. Although no previous work has confirmed that ts11MG invasively colonizes the liver, these current results suggest that this may be a possibility. The absence of any adverse effect of the ts11MG/10 + FMG/45 treatment on HCT and STP may also indicate that a ts11MG vaccination is useful in reducing possible stress responses to FMG inoculations given during the prelay period.

To our knowledge, this is the first report that has examined the possible effects of MGBac vaccinations on the blood characteristics of commercial layers. More specifically, this is the first report that has examined various blood parameter responses to a prelay MGBac vaccination alone or in combination with ts11MG, with or without a subsequent FMG vaccination overlay during lay. The current results of this study alone suggest that a prelay MGBac vaccination does not contribute any added identifiable effects on the commercial layer blood parameters examined at the ages specified in this study. Previous results in earlier companion reports have likewise shown that a prelay MGBac vaccination alone or in combination with ts11MG has no subsequent adverse effects on the performance characteristics (Jacob et al., 2014) or internal egg and eggshell characteristics (Jacob et al., 2015) of commercial layer chickens. In conclusion, a prelay vaccination regimen using ts11MG alone or in combination with MGBac may be used to protect commercial laying hens against possible field-strain MG infections without imposing any consequential effects on their HCT or on their circulating lipid and protein concentrations.

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