Effects of time-specific F-strain *Mycoplasma gallisepticum* inoculation overlays on prelay ts-11-strain *M. gallisepticum* vaccination on blood characteristics of commercial laying hens[1,2]

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ABSTRACT Two trials were conducted to determine the effects of a prelay ts-11-strain *Mycoplasma gallisepticum* (ts-11MG) vaccination alone or in combination with subsequent time-specific F-strain *M. gallisepticum* (FMG) inoculations on the blood characteristics of commercial laying hens. The following 4 treatments were utilized: 1) sham vaccination at 10 wk of age, 2) vaccination of ts-11MG at 10 wk, 3) ts-11MG at 10 wk overlaid by FMG inoculation at 22 wk, and 4) ts-11MG at 10 wk overlaid by FMG at 45 wk. Parameters measured in both trials were whole blood hematocrit, plasma protein, serum cholesterol, serum triglycerides, and serum calcium. No significant age × treatment interactions and no significant age or treatment main effects were observed for any of the blood parameters investigated, except for serum calcium. At wk 22, serum calcium concentrations were increased by vaccination with ts-11MG at 10 wk, and levels were further increased when the ts-11MG vaccination at 10 wk was overlaid by an FMG inoculation at 22 wk. These results suggest that ts-11MG vaccination at 10 wk of age alone or combined with F-strain inoculum overlays at either 22 or 45 wk may be used without any consequential effects on hematocrit or the lipid and protein levels in the blood of commercial layers. Because elevations in serum calcium were not associated with changes in hen performance, as reported in a previous companion article, it is further suggested that prelay ts-11MG vaccination before FMG inoculation overlays during lay may provide adequate protection against field strain *M. gallisepticum* infections while being innocuous to layer performance.

Key words: blood, commercial layer, F-strain *Mycoplasma gallisepticum*, ts-11-strain *Mycoplasma gallisepticum*

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INTRODUCTION

Vaccine programs have been implemented by egg producers maintaining multi-age layer facilities to prevent losses from *Mycoplasma gallisepticum* (**MG**; Carpenter et al., 1981; Mohammed et al., 1987; Kleven, 1998). Three live strains of MG have been licensed for use to help prevent and control MG outbreaks (Evans and Hafez, 1992; Ley and Yoder, 1997). The F-strain of MG (**FMG**) has predominantly been used because of its proven ability to not only protect layers from field strains but also to displace more virulent field strains of MG (Levisohn and Kleven, 1981). The F-strain of MG also has a lower bird-to-bird transmission rate (Kleven et al., 1990).

The F-strain of MG, however, is not completely apathogenic (Lin and Kleven, 1982), and the spread to MG-free turkey and chicken farms has been reported (Ley et al., 1993; Turner and Kleven, 1998). Inoculation of pullets with FMG at 12 wk of age has been reported to lead to a delay in egg production (**EP**) and to a decrease in total EP (Burnham et al., 2002). Furthermore, Branton et al. (1988) reported a significant reduction in EP subsequent to the inoculation of 45-wk-old commercial layers with FMG.

Vaccine strains of MG include the ts-11 (**ts-11MG**) and 6/85 (**6/85MG**) strains. These are apathogenic and are considered to be safer than FMG with little to no potential of spreading from bird to bird (Levisohn and Kleven, 1981; Kleven et al., 1990). The ts-11- and 6/85-strain vaccines have not proven to displace wild-type MG (Kleven, 1998). Furthermore, these strains may not confer continued protection as does the F-

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strain throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987). The effects of ts-11MG vaccination on the performance (Vance et al., 2008a) and internal egg and eggshell characteristics (Vance et al., 2008b) in layers have been described. Differential leukocyte counts of birds after exposure to *Mycoplasma* species have also been described (Kerr and Olson, 1970; Branton et al., 1997); however, further characterization studies of the blood from birds infected with FMG (Burnham et al., 2003) and 6/85MG (Peebles et al., 2008) have just recently been initiated, with no literature available for blood characteristics when birds are vaccinated with ts-11MG. Burnham et al. (2003) suggested that the effects of an FMG inoculation on EP were associated with changes in whole-blood hematocrit (**HCT**) and that concentration changes in serum triglycerides (**STRIG**) and total plasma protein (**PP**) may be indicative of the influence of FMG on liver metabolism. Peebles et al. (2008) also showed that an FMG inoculation overlaid on a previous 6/85MG vaccination may increase serum calcium (**SCA**) concentrations without any associated effects on HCT, STRIG, PP, or serum cholesterol (**SCHOL**) concentrations.

More testing is needed to determine if combinations of vaccines can lessen the impact of prelay FMG vaccination on layer blood characteristics. Therefore, the objective of the current study was to determine the effects of prelay ts-11MG vaccinations and time-specific FMG inoculation overlays administered during lay at 22 (approximate onset of lay) and 45 wk of age on those blood parameters that were examined by Burnham et al. (2003) and Peebles et al. (2008) in commercial laying hens.

MATERIALS AND METHODS

Bird Management

Two trials were performed, during similar months in 2 consecutive years, using Hy-Line W-36 pullets that were obtained at 1 d of age from a commercial source that was monitored and certified free of both MG and *Mycoplasma synoviae* (**MS**; USDA-Animal and Plant Health Inspection Service-Veterinary Services, 2003). Until 10 wk of age, birds were raised, vaccinated, and tested for the presence of MG and MS as described by Vance et al. (2008a).

At 10 wk of age, 11 pullets were randomly selected and placed in each of 16 negative-pressure fiberglass biological isolation units in each trial, with 4 replicate units assigned to each of 4 treatments. Hen numbers were reduced to 10 per unit at point of lay (22 wk of age). The testing of birds during lay for the presence of MG and further details of layer management are provided by Vance et al. (2008a). Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. The ingredient percentages and the calculated and determined analyses of the diets were as described by Burnham et al. (2002). No medications were administered during either trial. Both trials were conducted under an approved USDA Animal Care and Use protocol.

ts-llMG and FMG Inoculation

Birds in treatment 1 (control) received no MG inoculation but were sham-vaccinated via eye drop in the right eye with 0.04 mL of sterile Frey's media (Frey et al., 1968) at 10 wk of age. Treatment 2 contained birds that were administered 0.04 mL of ts-11MG vaccine (Merial Select Inc., Gainesville, GA) via eye drop in the right eye, at 10 wk of age (ts-11/10). The ts-11MG vaccine was obtained frozen and was thawed according to the specifications of the manufacturer before use. Birds belonging to treatment 3 received the wk 10 ts-11MG vaccination followed by a 0.04-mL overlay inoculation of FMG (99th passage above the unknown level) via eye drop in the left eye at 22 wk (ts-11/10-F/22). Treatment 4 consisted of birds given the wk 10 ts-11MG vaccination followed by an overlay inoculation of FMG similar to that in treatment 3, but at 45 wk of age (ts-11/10-F/45). The FMG culture was advanced after being received from S. H. Kleven (University of Georgia, Athens). The ts-11MG vaccine and FMG inoculum titers administered at their respective times in each trial are described by Vance et al. (2008b).

Data Collection

In each trial, 2 tagged hens per replicate isolation unit were bled from the left cutanea ulnae wing vein. Blood was drawn and harvested at the same time of day at 22, 24, 32, 43, and 56 wk of age, and data collected at wk 22, 24, 32, and 43 were designated as belonging to age interval I, whereas data collected wk 56 was designated as belonging to interval II. At wk 22, birds were vaccinated with FMG and subsequently bled on the same day. In age interval I, 8 replicate units were assigned to the ts-11/10 treatment until birds in 4 of those units were inoculated with FMG at 45 wk and assigned to the ts-11/10-F/45 treatment. Variables measured in both trials were HCT and PP, SCHOL, STRIG, and SCA concentrations.

Analyses of Blood and Serum Constituents

Hematocrit was expressed as percentage of blood packed cell (primarily red blood cell) volume and was determined through the use of capillary tubes that were centrifuged in a micro-HCT centrifuge and then read with a microcapillary reader. Serum cholesterol and STRIG expressed in milligrams per deciliter and PP expressed in grams per deciliter were determined by placing 10 μL of serum or plasma for each test on test slides, which were analyzed on a Kodak Ektachem DT-60 analyzer (Eastman Kodak Co., Rochester, NY) as described by Latour et al. (1996). Similarly, SCA concentrations expressed in milligrams per deciliter were

determined by placing 10 μL of serum on a test slide, which was analyzed on a Kodak Ektachem DTSC module analyzer (Eastman Kodak Co.), according to procedures of Tietz (1986). Control analyses were performed to assure that each sample was in the appropriate test range for accurate analysis.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. Data from wk 22 to 43 (age interval I) and at wk 56 (age interval II) were analyzed separately. The data of both trials were pooled and then analyzed together. Therefore, the results from both trials were not reported independently but were reported over both trials. Trial was considered as a random effect. All data within age interval I were subjected to a repeated measures analysis. Data in age interval II were subjected to 1-way ANOVA. In the first age interval, control, ts-11/10, and ts-11/10-F/22 inoculation treatments were compared. In the second age interval, control, ts-11/10, ts-11/10-F/22, and ts-11/10-F/45 treatment groups were compared. Individual sample data within each replicate unit were averaged before analysis. Least squares means were compared in the event of significant global effects (Steel and Torrie, 1980). Global effects and differences among least squares means were considered significant at $P \leq 0.05$. All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

As described previously in a companion article by Vance et al. (2008a) in which these same birds and treatments were used, control birds remained *Mycoplasma*-free (negative for both MG and MS), whereas systemic infections were confirmed in MG-treated birds, whether the treatment was with the ts-11MG or FMG. Vance et al. (2008a) also found that mortality and EP were not adversely affected by the inoculation treatments administered. For numerical reference, Vance et al. (2008a) showed that no mortalities were due to treatment and that total EP across lay (wk 22 to 56) was 76.8, 76.8, 70.8, and 72.2% for the control, ts-11/10, ts-11/10-F/22, and ts-11/10-F/45 treatments, respectively.

There was a significant ($P \leq 0.05$) age × treatment interaction for SCA in age interval I (Table 1). At wk 22, SCA was higher in the ts-11/10-F/22 treatment compared with that in the ts-11/10 treatment, and SCA in both the ts-11/10-F/22 and ts-11/10 treatments were higher than that in the control group. The SCA of the birds at wk 22 was not only increased by the use of ts-11MG at 10 wk of age but was further elevated by an overlay of FMG at 22 wk. The observation of a significant effect of the wk 10 ts-11MG vaccination on SCA only initially at 22 wk and the absence of any subsequent effect at wk 24, 32, and 43 suggests that ts-11MG may influence SCA for only a limited length of time (12 wk) after inoculation. However, because FMG inoculation and blood collection at wk 22 were performed on the same day, the additional influence of the FMG inoculation on SCA at wk 22 may have occurred in response to the stress of the inoculation procedure itself rather than the FMG. Peebles et al. (2008) have also shown changes in SCA after the use of 6/85MG prelay. However, in that study, SCA at wk 47 in layers that previously received 6/85MG at 10 wk followed by FMG at 45 wk was increased relative to sham controls and those that received 6/85MG at 10 wk followed by FMG at 22 wk. The contrast in the results of this study with that of Peebles et al. (2008) would indicate that when overlaid with FMG at wk 45, a prelay 6/85MG vaccination exerts an effect on SCA in postpeak lay that ts-11MG does not. Nevertheless, in companion articles in which the eggshell characteristics of the birds in the current study were previously reported (Vance et al., 2008a,b), it was noted that none of the inoculation treatments used, in which ts-11MG was given alone or in conjunction with FMG during lay, had any effect on eggshell breaking strength, eggshell weight per unit of surface area, or percentage of shell weight of the eggs of the hens. The increase in SCA at wk 22 observed in the current study, therefore, had no subsequent impact on eggshell quality. The change in SCA after the 6/85MG and FMG inoculation regimens reported by Peebles et al. (2008), likewise, had no impact on eggshell quality (Viscione et al., 2008).

Significant increases in PP have been shown to be indicative of dehydration in broilers (Warriss et al., 1997). Furthermore, in a review of the literature, Boyd (1981) has documented that PP commonly exhibits a greater increase than HCT during dehydration. In trials conducted by Burnham et al. (2003), in which birds were inoculated with FMG at 12 wk of age and maintained under the same aforementioned conditions, HCT and PP increased 8 and 10 wk, respectively, after challenge. Increases in these 2 individual blood parameters between 8 and 10 wk postchallenge were suggestive of a dehydration response to the F-strain inoculation. The HCT levels in FMG-inoculated birds in that study returned to those of the controls after wk 20 and PP levels were subsequently depressed at wk 52, indicating that the birds adjusted through other physiological means to the stress of the F-strain inoculation.

The birds that experienced increases in HCT and PP in response to a prelay FMG inoculation (Burnham et al., 2003) also exhibited a subsequent delay in onset of lay and a decrease in total EP (Burnham et al., 2002). In the present study, in which ts-11MG vaccination was given at 10 wk and FMG was given as an overlay inoculation at either 22 or 45 wk, no significant treatment effects were observed for HCT or PP at any of the times investigated. Vance et al. (2008a) have also noted that except for a singular effect on egg weight at only 1 time period, the ts-11/10 treatment had no impact on layer performance. The use of ts-11MG rather than FMG in

Table 1. Serum calcium concentrations in control, ts-11-strain *Mycoplasma gallisepticum* at 10 wk (ts-11/10), and ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 22 wk (ts-11/10-F/22) treatment groups at 22, 24, 32, and 43 wk of age (age interval I)[1,2,3]

	Week of age			
Treatment	22	24	32	43
	(mg/dL)			
Control	20.3[c]	23.0	21.9	25.0
ts-11/10	30.6[b]	22.1	23.7	30.4
ts-11/10-F/22	39.8[a]	20.4	24.4	29.1

[a–c]Means within the wk 22 column with no common superscript differ significantly ($P \leq 0.05$).

[1]Within each column (week), 2 samples from 8 replicate isolation units for the control and ts-11/10-F/22 treatments and 16 replicate isolation units for the ts-11/10 treatment were used for means calculations.

[2]SEM based on pooled estimate of variance = 3.07.

[3]There were no significant differences between treatment means at wk 24, 32, and 43 ($P > 0.05$).

prelay pullets may, therefore, eliminate a subsequent dehydration effect and an accompanying decline in the performance of laying hens. Furthermore, when comparing the HCT results in this study to the aforementioned study by Burnham et al. (2003), it appears that the 10 wk ts-11 vaccination may have prevented the stress response to either of the subsequent F-strain inoculations during lay.

More recently, Peebles et al. (2008) additionally reported the effects of 6/85MG on layer blood characteristics. When inoculated prelay at 10 wk, as in this study, 6/85MG had no effect on HCT but elevated PP at 32 wk of age. The 6/85 and ts-11 strains of MG are considered apathogenic and safer than FMG. Similar to the wk 10 ts-11MG vaccination, a 10 wk 6/85MG vaccination has also been shown to exert no impact on layer performance (Viscione et al., 2008). However, unlike the 6/85MG vaccination, the ts-11MG vaccination had no subsequent effects on either HCT or PP in layers. The use of ts-11MG as a prelay vaccine rather than 6/85MG avoided a subsequent elevation in PP during lay. This suggests that the ts-11MG vaccination may be milder than that of 6/85MG in regards to its impact on the PP of layers.

Colonization of the liver by FMG may negatively affect liver metabolism (Sahu and Olson, 1976), and an elevation in STRIG is known to be a common response to the presence of infectious disease agents (Guyton and Hall, 1996). Under the same experimental conditions, Burnham et al. (2003) reported significant changes in HCT, PP, and STRIG during lay when a prelay FMG inoculation was given alone. Furthermore, in a study by Peebles et al. (2007), it was shown that PP at 34 wk of age was higher in birds that were inoculated with FMG at the onset of lay compared with those that were inoculated prelay. For PP, STRIG, and SCHOL in the present study, there were no significant age × treatment interactions and no significant age or treatment main effects in age interval I, and there was no significant effect due to treatment in interval II (wk 56). There was also no significant effect due to treatment for SCA in interval II. This further suggests that the 10 wk ts-11MG vaccination may help prevent alterations in HCT, PP, or STRIG that might occur from subsequent FMG inoculations given either at 22 or 45 wk of age. For reference, the treatment means for HCT, PP, STRIG, and SCHOL in interval I and for HCT, PP, SCA, STRIG, and SCHOL in interval II are provided in Tables 2 and 3, respectively.

In conclusion, the results of this and previous studies suggest that the ts-11MG and FMG treatment combinations may help provide continual protection against field-strain MG infections and may overcome some of the adverse effects that FMG may have if given alone. The prelay administration of ts-11MG also appears even more suitable than that of 6/85MG as well as FMG when the blood and associated performance characteristics of the birds are considered. The MG strain

Table 2. Hematocrit (HCT) and concentrations of plasma total protein (PP), serum triglycerides (STRIG), and serum cholesterol (SCHOL) in control, ts-11-strain *Mycoplasma gallisepticum* at 10 wk (ts-11/10), and ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 22 wk (ts-11/10-F/22) treatment groups in age interval I (across 22, 24, 32, and 43 wk)[1,2]

Treatment	HCT (%)	PP (g/dL)	STRIG (mg/dL)	SCHOL (mg/dL)
Control	26.9	5.21	2,501	193
ts-11/10	26.9	5.20	2,592	168
ts-11/10-F/22	27.2	4.79	2,735	172
Pooled SEM	0.83	0.623	396.0	13.6

[1]Within each column (parameter), 2 samples from 32 replicate isolation units for the control and ts-11/10-F/22 treatments and 64 replicate isolation units for the ts-11/10 treatment were used for means calculations.

[2]*P*-values for HCT, PP, STRIG, and SCHOL were 0.58, 0.44, 0.72, and 0.23, respectively.

Table 3. Hematocrit (HCT) and concentrations of plasma total protein (PP), serum calcium (SCA), serum triglycerides (STRIG), and serum cholesterol (SCHOL) in control, ts-11-strain *Mycoplasma gallisepticum* at 10 wk (ts-11/10), ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 22 wk (ts-11/10-F/22), and ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 45 wk (ts-11/10-F/45) treatment groups in age interval II (56 wk)[1,2]

Treatment	HCT (%)	PP (g/dL)	SCA (mg/dL)	STRIG (mg/dL)	SCHOL (mg/dL)
Control	25.1	3.76	26.2	2,698	142
ts-11/10	23.8	4.48	23.1	2,041	139
ts-11/10-F/22	24.3	4.18	23.1	2,213	142
ts-11/10-F/45	24.5	3.96	27.1	2,862	141
Pooled SEM	3.14	0.300	1.81	389.1	16.0

[1]Within each column (parameter), 2 samples from 8 replicate isolation units for each treatment were used for means calculations.

[2]*P*-values for HCT, PP, SCA, STRIG, and SCHOL were 0.38, 0.40, 0.28, 0.41, and 0.99, respectively.

combinations and sequences of administration used in this study show promise as research continues to develop new and improved protocols to better protect flocks from field-strain MG infections.

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