

Effects of Prelay 6/85-Strain *Mycoplasma gallisepticum* Inoculation Alone or in Conjunction with the Inoculation of F-Strain *Mycoplasma gallisepticum* During Lay on the Blood Characteristics of Commercial Egg-Laying Hens^{1,2}

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ABSTRACT The effects of 6/85-strain *Mycoplasma gallisepticum* (6/85MG) inoculation alone or in conjunction with F-strain *M. gallisepticum* (FMG) overlays and their timing on the blood characteristics of commercial egg-laying hens were investigated. Control birds received sham inoculations at 10 wk of age. Birds in a second treatment group were inoculated with 6/85MG at 10 wk of age, those in a third treatment group were inoculated with 6/85MG at 10 wk followed by an overlay inoculation of FMG at 22 wk, and those in a fourth treatment group were inoculated with 6/85MG at 10 wk followed by an overlay inoculation of FMG at 45 wk. Parameters investigated at 24, 32, 43, and 47 wk were hematocrit, plasma total protein, and serum calcium,

triglycerides, and cholesterol. No significant treatment effects were noted for hematocrit, serum triglycerides, or serum cholesterol. However, at wk 32, plasma protein was greater in birds that received 6/85MG at 10 wk or 6/85MG at 10 wk and FMG at 22 wk in comparison to controls. Also, at wk 47, serum calcium concentration was greater in birds that received 6/85MG at 10 wk and FMG at 45 wk compared with controls and those that received 6/85MG at 10 wk and FMG at 22 wk. These results suggest that the prelay inoculation of pullets with 6/85MG may subsequently elevate plasma protein, and in conjunction with an FMG overlay at 45 wk, may increase serum calcium concentrations in laying hens.

Key words: blood, F-strain *Mycoplasma gallisepticum*, inoculation, 6/85-strain *Mycoplasma gallisepticum*

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INTRODUCTION

Inoculation of pullets with F-strain *Mycoplasma gallisepticum* (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). However, there are possible benefits of using a prelay 6/85-strain *M. gallisepticum* (6/85MG) inoculation in conjunction with FMG inoculations during lay to effectively protect flocks against field-strain *M. gallisepticum* (MG) infections while reducing possible subsequent negative effects due to a prelay FMG inoculation. Furthermore, commercial operations may acquire flocks already in-

culated with 6/85MG but may desire to continue using the FMG inoculation regimen familiar to them.

Inoculation of 6/85MG at 10 wk followed by an overlay of FMG (i.e., subsequent FMG inoculation during lay in combination with a prelay 6/85MG inoculation) at 22 wk has been shown to decrease yolk:albumen ratio (Viscione et al., 2008b), increase yolk moisture content (Viscione et al., 2008a), and decrease yolk linolenic acid concentration (Viscione et al., 2008a) in layers. Furthermore, Burnham et al. (2003a) suggested that alterations in EP in response to a 12-wk FMG inoculation may be associated with changes in whole-blood hematocrit (HCT) and that postpeak decreases in serum triglycerides (STRIG) and plasma total protein (PP) levels after the inoculation of FMG at 12 wk may be the result of a chronic effect of FMG on hepatic lipid and protein synthesis. Using the S6-strain of MG (S6MG; a more virulent strain of MG than the 6/85- and F-strains), Peebles et al. (2006) additionally noted that inoculation of S6MG at 10 wk of age increased HCT at 20 wk and serum calcium (SCA) across wk 47 and 58 and that S6MG inoculations at 10 or 22 wk of age elevated SCA across wk 24, 32, and 43.

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Table 1. Hematocrit (HCT) and concentrations of serum calcium (SCA), triglycerides (STRIG), and cholesterol (SCHOL) in control, 6/85-strain *Mycoplasma gallisepticum* (6/85MG) at 10 wk (6/85MG-10), and 6/85MG at 10 wk and F-strain *M. gallisepticum* at 22 wk (6/85MG-10, FMG-22) treatment groups in age interval I (across 24, 32, and 43 wk)¹

Treatment	HCT ² (%)	SCA ³ (mg/dL)	STRIG ⁴ (mg/dL)	SCHOL ⁵ (mg/dL)
Control	27.2	25.9	2,588	158
6/85MG-10	26.9	28.7	3,039	173
6/85MG-10, FMG-22	27.4	26.4	2,626	168

¹n = 12 replicate isolation units, containing 10 birds each, were used for calculation of treatment means within each column (parameter).

²SEM based on pooled estimate of variance = 0.582.

³SEM based on pooled estimate of variance = 2.13.

⁴SEM based on pooled estimate of variance = 210.

⁵SEM based on pooled estimate of variance = 11.0.

Based on the results of these previous studies that determined the effects of prelay inoculations of FMG and S6MG on the blood characteristics of layers, it was hypothesized that a prelay 6/85MG inoculation alone or in conjunction with FMG inoculations during lay might also alter HCT, STRIG, PP, or SCA in layers. Therefore, the goal of this study was to investigate the effects of a prelay 6/85MG inoculation overlaid with FMG inoculations during lay (22 or 45 wk of age) on the blood characteristics of commercial layers. Based on a report by Branton et al. (1988), showing a significant reduction in EP subsequent to the inoculation of 45-wk-old commercial layers with FMG, an FMG inoculation overlay at 45 wk was tested in addition to an FMG inoculation overlay at the onset of lay (22 wk).

MATERIALS AND METHODS

Bird Management

In both trials, 1-d-old Hy-Line W-36 Leghorn pullets were obtained from a commercial hatchery certified free of MG and *Mycoplasma synoviae* (USDA-APHIS-VS, 2003). Until 10 wk of age, birds were raised, vaccinated, and tested for the presence of MG and *M. synoviae* as described by Viscione et al. (2008b). At 10 wk of age, 11 pullets were randomly assigned to each of 16 negative-pressure isolation units, with 4 units assigned to each of 4 treatments. At initiation of lay, the number of hens in each treatment unit was decreased to 10. In trial II, the location of treatments within the isolation facility was different from that in trial I to assure environmental randomization between trials. The testing of layers for the presence of MG and further details of layer housing and management are provided by Viscione et al. (2008b). Pullet and layer diets met or exceeded National Research Council (1994) recommendations. The ingredient percentages and the calculated and determined analyses of those diets were as described by Burnham et al. (2002). Both trials were conducted under an approved USDA animal care and use protocol.

6/85MG and FMG Inoculation

Control birds (control) received sham eye drop inoculations in the right eye with 0.04 mL of sterile Frey's broth media at 10 wk of age. A second treated group of birds were spray-inoculated with 6/85MG (Noblis MG 6/85, Intervet Inc., Millsboro, DE) at 10 wk of age (6/85MG-10). In a third treatment group, birds inoculated with 6/85MG at 10 wk received a 0.04 mL overlay eye drop inoculation of FMG (99th passage above the unknown level) in the right eye at 22 wk (6/85MG-10, FMG-22); and a fourth treatment group was inoculated with 6/85MG at 10 wk followed by a 45-wk overlay inoculation of FMG (6/85MG-10, FMG-45). The FMG culture was advanced after being received from S. H. Kleven (University of Georgia, Athens). Titers of the inocula are provided by Viscione et al. (2008b).

Data Collection

In both trials, blood samples from 2 tagged birds from each replicate unit were collected at 24, 32, 43, and 47 wk of age for determination of HCT, SCA, STRIG, serum cholesterol (SCHOL), and PP concentrations. All data collected at wk 24, 32, and 43 were designated as belonging to age interval I, and data collected at wk 47 were designated as belonging to interval II.

Blood and Serum Constituents

Two tagged birds from each unit were selected and bled from the cutanea ulnae wing vein. Blood (4 to 5 mL) was collected into tubes designed for either serum or plasma collection. Two heparinized capillary tubes (75 mm long) were used on each blood sample for HCT determination. Serum and plasma tubes were centrifuged at 5,000 × g for 20 min, and serum and plasma samples were stored at -20°C for later analysis. A Kodak Ektachem DT-60 analyzer (Eastman Kodak Co., Rochester, NY) was used to determine levels of STRIG, SCHOL, and PP. A Kodak Ektachem DTSC analyzer (Eastman Kodak Co.) was used to assay serum sam-

Table 2. Hematocrit (HCT) and concentrations of plasma total protein (PP), serum triglycerides (STRIG), and serum cholesterol (SCHOL) in control, 6/85-strain *Mycoplasma gallisepticum* (6/85MG) at 10 wk (6/85MG-10), 6/85MG at 10 wk and F-strain *M. gallisepticum* (FMG) at 22 wk (6/85MG-10, FMG-22), and 6/85MG at 10 wk and FMG at 45 wk (6/85MG-10, FMG-45) treatment groups in age interval II (47 wk)¹

Treatment	HCT ² (%)	PP ³ (g/dL)	STRIG ⁴ (mg/dL)	SCHOL ⁵ (mg/dL)
Control	27.4	5.65	2,505	183
6/85MG-10	28.4	5.06	2,555	189
6/85MG-10, FMG-22	26.5	5.23	2,068	207
6/85MG-10, FMG-45	27.6	4.85	3,010	183

¹n = 4 replicate isolation units, containing 10 birds each, were used for calculation of treatment means within each column (parameter).

²SEM based on pooled estimate of variance = 0.865.

³SEM based on pooled estimate of variance = 0.656.

⁴SEM based on pooled estimate of variance = 699.

⁵SEM based on pooled estimate of variance = 18.6.

ples for SCA. Hematocrit was expressed as percentage blood packed cell volume. Concentrations of SCHOL, STRIG, and SCA were expressed as milligrams per deciliter, and PP was expressed in grams per deciliter.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. Data at wk 24, 32, and 43 (age interval I) were analyzed separately from data at wk 47 (age interval II). The data of both trials were pooled 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 because of data collection at multiple age periods within that interval. Data obtained in interval II were subjected to 1-way ANOVA because of data collection at only 1 age period (wk 47) within that interval.

In the first age interval, control; 6/85MG-10; and 6/85MG-10, FMG-22 inoculation treatment groups were compared. In the second age interval, control; 6/85MG-10; 6/85MG-10, FMG-22; and 6/85MG-10, FMG-45 groups were compared. Individual sample data within each of the treatment replicate units 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 indicated in a companion article by Viscione et al. (2008b), in which these same birds and treatments were used, control hens remained MG-negative, whereas MG-inoculated hens were MG-positive throughout the study. Furthermore, Viscione et al. (2008b) reported that these inoculation treatments had no influence on bird mortality, BW, or EP in any age interval and

that the 6/85MG-and FMG-inoculated hens exhibited no outward pathological symptoms.

Likewise, in the current study, no significant effects of any kind were noted for HCT, SCA, STRIG, or SCHOL in interval I, or for HCT, PP, STRIG, or SCHOL in interval II. Nevertheless, for reference, the treatment means for HCT, SCA, STRIG, and SCHOL in interval I and for HCT, PP, STRIG, and SCHOL in interval II are provided in Tables 1 and 2, respectively. However, there was a significant age \times treatment interaction ($P \leq 0.03$) for PP concentration in age interval I (Table 3), and there was a significant treatment main effect ($P \leq 0.04$) for SCA at wk 47 (interval II; Table 4). In control birds, PP was greater at wk 43 than at wk 24 and 32. Treatment differences in PP concentration were found only at wk 32 in interval I. At wk 32, PP concentrations were significantly greater for birds in the 6/85MG-10 and 6/85MG-10, FMG-22 treatment groups in comparison to the control group. At wk 47, SCA was greater in the 6/85MG-10, FMG-45 group compared with the control and 6/85MG-10, FMG-22 groups, with the 6/85MG-10 treatment intermediate.

Burnham et al. (2003a) concluded that decreased concentrations of lipids (i.e., STRIG) in the blood during lay in response to the inoculation of FMG at 12

Table 3. Plasma total protein concentration (g/dL) in control, 6/85-strain *Mycoplasma gallisepticum* (6/85MG) at 10 wk (6/85MG-10), and 6/85MG at 10 wk and F-strain *M. gallisepticum* at 22 wk (6/85MG-10, FMG-22) treatment groups at 24, 32, and 43 wk of age (age interval I)^{1,2}

Treatment	Week of age		
	24	32	43
Control	3.87 ^B	3.85 ^{B,b}	4.89 ^A
6/85MG-10	4.01	4.43 ^a	4.56
6/85MG-10, FMG-22	4.10	4.90 ^a	4.44

^{a,b}Means within week (column) with no common superscript differ significantly ($P \leq 0.05$).

^{A,B}Means within treatment (row) with no common superscript differ significantly ($P \leq 0.05$).

¹n = 4 replicate isolation units, containing 10 birds each, were used for calculation of treatment means within each column (wk).

²SEM based on pooled estimate of variance = 0.343.

Table 4. Serum calcium concentration in control, 6/85-strain *Mycoplasma gallisepticum* (6/85MG) at 10 wk (6/85MG-10), 6/85MG at 10 wk and F-strain *M. gallisepticum* (FMG) at 22 wk (6/85MG-10, FMG-22), and 6/85MG at 10 wk and FMG at 45 wk (6/85MG-10, FMG-45) treatment groups in age interval II (47 wk)^{1,2}

Treatment	Concentration (mg/dL)
Control	29.0 ^b
6/85MG-10	34.5 ^{ab}
6/85MG-10, FMG-22	29.4 ^b
6/85MG-10, FMG-45	36.0 ^a

^{a,b}Means with no common superscript differ significantly ($P \leq 0.05$).

¹n = 4 replicate isolation units, containing 10 birds each, were used for calculation of treatment means.

²SEM based on pooled estimate of variance = 1.96.

wk of age may be directly responsible for reductions in total yolk lipid and yolk cholesterol and changes in the yolk fatty acid profiles that were noted in an earlier companion report by Burnham et al. (2003b). Conversely, there were no significant changes in any of the blood lipids examined in this study after treatment with 6/85MG at 10 wk alone or in combination with FMG overlays at 22 or 45 wk of age, despite reported alterations in yolk palmitic, oleic, and linolenic acid levels by Viscione et al. (2008a), using these same birds and treatments. These results indicate that the association between the blood and yolk lipid responses to MG inoculation is influenced by the strain of MG used and the timing of inoculation. Burnham et al. (2003a) found no change in SCA after an FMG inoculation at 12 wk of age. Likewise, in the present study, SCA was not changed as the result of a 6/85MG inoculation at 10 wk. However, SCA did increase due to an FMG inoculation at 45 wk overlaid on a 6/85MG inoculation at 10 wk. Similarly, Peebles et al. (2006) noted that S6MG inoculations at 22 wk increased the subsequent SCA in layers. Therefore, these results would also indicate that the effects of an MG inoculation on SCA are influenced by the strain of MG and the age at which it's given.

It is noteworthy that both the 6/85MG-10 and 6/85MG-10, FMG-22 treatments significantly increased PP at wk 32, indicating that singular prelay (10 wk of age) 6/85MG inoculations may lead to increases in PP up to 22 wk later. This effect, nevertheless, was lost 33 wk later in association with a significant increase in PP in control birds at wk 43. Warris et al. (1997) suggested that significant increases in PP were indicative of dehydration in broilers. Although the treatments did not affect overall hen performance, including weekly and total EP, as shown in the companion report by Viscione et al. (2008b), these current results suggest that the prelay inoculation of 6/85MG caused a delayed dehydration effect during peak EP. However, this effect may later become ameliorated should age-related dehydration occur. Burnham et al. (2003a) reported that an FMG inoculation at 12 wk increased HCT at 20 wk as well as PP at 22 wk. In contrast, although

PP was elevated in response to the 6/85MG inoculation at 10 wk, no response in HCT was found. In a review of published data on PP and HCT during dehydration, Boyd (1981) stated that a greater increase in PP was usually observed. In comparison to the prelay 6/85MG inoculation used in the current study, the prelay FMG inoculation administered by Burnham et al. (2003a) appears to have exerted a stronger effect on the hydration statuses of the birds in that study, so that both PP and HCT became elevated.

Despite possible 6/85MG and FMG inoculation treatment and timing effects on the PP and SCA of birds housed in environmentally controlled conditions, these changes exerted no overall effect on layer performance, as confirmed in a previous companion article by Viscione et al. (2008b). These results establish that because of the lack of any concomitant treatment effects on bird performance including EP, prelay (10 wk of age) 6/85MG inoculations may be a suitable substitute for prelay FMG inoculations. The FMG overlays during lay on prelay 6/85MG inoculations may also provide continual protection against field-strain MG infections without eliciting any subsequent suppressive effects on performance. Because these birds were housed in isolation units, these results do not preclude the possibility that different or greater performance and physiological effects may occur in birds infected prelay, at lay onset, or late in lay with 6/85MG or FMG alone or in combination, when housed in facilities where there are increased levels of environmental stress.

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