Effects of 6/85-strain *Mycoplasma gallisepticum* vaccination alone at ten weeks of age or in conjunction with F-strain *Mycoplasma gallisepticum* inoculation overlays at twenty-two or forty-five weeks of age on the reproductive and digestive organs of commercial egg-laying hens[1,2]

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ABSTRACT Two trials were conducted to determine the effects of a prelay 6/85-strain *Mycoplasma gallisepticum* (6/85MG) vaccination alone or in conjunction with time-specific F-strain *M. gallisepticum* (FMG) inoculation overlays on the gross reproductive and digestive organ characteristics of commercial egg-laying hens. In each trial, the following 4 treatments were applied: 1) sham vaccination at 10 wk of age; 2) vaccination of 6/85MG at 10 wk; 3) 6/85MG at 10 wk overlaid by FMG inoculation at 22 wk; and 4) 6/85MG at 10 wk overlaid by FMG at 45 wk. Two birds per isolation pen (experimental replicate unit) were necropsied at the end of both trials to observe the effects of treatment on liver weight, liver lipid and moisture concentrations, incidence of fatty liver hemorrhagic syndrome, ovary weight, mature ovarian follicle numbers, and the total and segmental weights, lengths, and histologies of the oviduct and small intestine. The applied treatments affected only liver moisture. Liver moisture content was greater in birds vaccinated with 6/85MG at 10 wk alone or in conjunction with FMG at 45 wk in comparison with sham vaccinated controls and birds that received a 6/85MG vaccination at 10 wk overlaid by an FMG inoculation at 22 wk. Prelay 6/85MG vaccinations may be a suitable substitute for prelay FMG inoculations, and FMG overlays during lay on prelay 6/85MG vaccinations may also provide continual protection against field-strain MG infections without eliciting any subsequent suppressive effects on performance, as noted in an earlier study.

Key words: digestive organ, F-strain *Mycoplasma gallisepticum*, inoculation, reproductive organ, 6/85-strain *Mycoplasma gallisepticum*

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INTRODUCTION

Vaccination programs have been used on multi-age layer facilities to prevent production losses due to *Mycoplasma gallisepticum* (**MG**) infections (Carpenter et al., 1981; Mohammed et al., 1987). The F-strain of MG (**FMG**) has predominately been used because it can protect layers by its displacement of more virulent strains of MG (Levisohn and Kleven, 1981). Nevertheless, the prelay inoculation of pullets with FMG at 12 wk of age has been shown to decrease total egg production (**EP**) and to delay the onset of lay by as much as 1 wk (Burnham et al., 2002b). The 6/85 vaccine strain of MG (**6/85MG**) is apathogenic and is considered to be safer than FMG with negligible bird-to-bird transmission (Levisohn and Kleven, 1981; Kleven et al., 1990). Although the prelay administration of 6/85MG is known to disrupt layer EP less than FMG, FMG better protects flocks from the more virulent strains of MG (Kleven, 1998; Branton et al., 2002). Therefore, there are possible benefits of using a prelay 6/85MG vaccination in conjunction with FMG inoculations during lay.

The prelay vaccination of pullets with 6/85MG has recently been demonstrated to exert no suppressive effects on layer performance (Viscione et al., 2008b), but to elevate plasma protein levels during lay (Peebles et al., 2008), which was suggestive of a dehydration effect. Also, when prelay 6/85MG vaccinations were used in conjunction with FMG overlay inoculations at 45 wk, they resulted in increased serum calcium concentrations during lay (Peebles et al., 2008), and when used in conjunction with FMG overlay inoculations at 22

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wk, they resulted in reduced yolk/albumen ratios (Viscione et al., 2008b), increased yolk moisture contents (Viscione et al., 2008a), and decreased yolk linolenic acid concentrations (Viscione et al., 2008a). However, no information is available concerning the associated effects of these treatment regimens on the digestive and reproductive organ characteristics of layers. Therefore, the goal of this study was to investigate the effects of a prelay 6/85MG vaccination alone or in conjunction with FMG inoculations during lay (22 or 45 wk of age) on the reproductive and digestive organ characteristics of commercial layers.

MATERIALS AND METHODS

Bird Management and Treatment

One-day-old Hy-Line W-36 Leghorn pullets used in each of 2 trials were obtained from a commercial hatchery certified free of MG and *Mycoplasma synoviae* (**MS**; USDA-Animal and Plant Health Inspection Service-Veterinary Services, 2003), and both trials were subsequently conducted under an approved USDA Animal Care and Use protocol. Bird management and housing during the pullet and layer periods were as described by Viscione et al. (2008b). Nutrient levels in pullet and layer diets met or exceeded National Research Council (1994) recommendations. The ingredient percentages and the calculated and determined analyses of the diets were as described by Burnham et al. (2002b). In each trial, there were 10 hens assigned to each of 16 negative pressure isolation units, with 4 units assigned to each of 4 treatment groups.

Control birds received sham eye drop vaccinations in the right eye with 0.04 mL of sterile Frey's broth media (Frey et al., 1968) at 10 wk of age. A second treated group of birds was administered 0.04 mL of 6/85MG vaccine (Noblis MG 6/85, Intervet Inc., Millsboro, DE), via eye drop in the right eye, at 10 wk of age (**6/85MG-10**). In a third treatment group, birds vaccinated with 6/85MG at 10 wk received a 0.04-mL eye drop overlay inoculation of FMG (99th passage above the unknown level) in the left eye at 22 wk (**6/85MG-10, FMG-22**), and a fourth treatment group was vaccinated 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 6/85MG vaccine and FMG inoculum administered at their respective times in each trial are provided by Viscione et al. (2008b).

Data Collection

At the end of each trial (wk 58), 2 birds from each replicate unit were killed by cervical dislocation following an overnight (12 h) fast. Subsequently, the birds were weighed and select internal organs harvested. The following parameters were determined: liver weight; liver moisture and lipid concentrations; incidence of fatty liver hemorrhagic syndrome (**FLHS**); ovary weight and mature follicle numbers; total oviduct weight and length; weights, lengths, and histologies of the infundibulum, magnum, isthmus, uterus, and vagina; total small intestine weight and length; and weights, lengths, and histologies of the duodenum, jejunum, and ileum. Liver, ovary, and total oviduct and small intestine weights were calculated as percentages of BW. Furthermore, oviduct and small intestine segment weights were calculated as percentages of BW and total organ weight, and oviduct and small intestine segment lengths were calculated as percentages of total organ length. The number of mature yellow follicles ($\geq$12 mm in diameter) in an ovary was assigned a number from 0 to 5. Ovaries not having any mature follicles were assigned a 0, whereas the maximum number assigned for mature yellow follicles was 5 (Burnham et al., 2002a). Absent (normal), moderate, and severe descriptions for incidences of FLHS were assigned numbers 1, 2, and 3, respectively, by an individual observer.

Liver Moisture and Lipid Analysis

For analysis of liver moisture content, fresh liver samples (2 g) were dried according to the procedure of Peebles et al. (1999) in a commercial oven (Model EL20, General Electric Co., Chicago Heights, IL). Liver moisture content was calculated as the difference between the fresh and dry weights of the sample and was expressed as a percentage of fresh liver sample weight. For analysis of liver lipid content, lipid was extracted from fresh liver samples (3 g) according to the procedure described previously by Bligh and Dyer (1959) and as modified by Latour et al. (1998). Liver lipid content was expressed as a percentage of total fresh liver sample weight.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. 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. Data at 58 wk of age were subjected to one-way ANOVA to test for the effects of treatment. 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-squared 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 noted in a companion article (Viscione et al., 2008b), all initial tests confirmed that pullets were free

Table 1. Percentages of ovary weight (OVAW), oviduct weight (OVIW), and small intestine weight (SIW) in control, 6/85MG at 10 wk (6/85MG-10), 6/85MG at 10 wk and 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 at 58 wk of age[1]

Treatment	OVAW	OVIW	SIW
		(%)	
Control	2.90	4.16	1.66
6/85MG-10	2.80	4.03	1.54
6/85MG-10, FMG-22	2.70	3.61	1.48
6/85MG-10, FMG-45	2.63	4.02	1.45
Pooled SEM	0.16	0.29	0.13

[1]In each of 2 trials, 4 replicate isolation units, with 2 birds sampled from each unit, were used for calculation of treatment means.

of MG and MS and that control birds remained free of MG and MS throughout each trial, whereas those birds vaccinated with 6/85MG with or without FMG inoculation overlays tested positive for MG and negative for MS. Furthermore, the 6/85MG and FMG inoculated hens exhibited no outward pathological symptoms, and there were no treatment effects on mortality.

It has been previously reported by Burnham et al. (2002b) that weekly EP was delayed 1 wk and that total EP was reduced in layers inoculated with the FMG at 12 wk of age. Burnham et al. (2002a) later suggested that alterations in the performance and egg characteristics of the layers examined were related to mutual functional disturbances in the liver, ovary, and oviduct. This suggestion was supported by results showing a reduction in mature ovarian follicle numbers, decreases in the isthmal and vaginal proportions of the oviduct, and increased incidences of FLHS in birds that demonstrated depressed performance subsequent to FMG inoculation at 12 wk of age. However, the present results showed that the prelay vaccination of 6/85MG alone or in conjunction with FMG inoculation at 22 or 45 wk of age had no effect on FLHS incidence, liver histology, or on the same determined characteristics of the ovary and oviduct. Viscione et al. (2008b) also established earlier that FMG overlays at 22 or 45 wk on 6/85MG vaccinations at 10 wk caused no adverse effects on performance, including weekly and total EP. This suggests that the prelay use of 6/85MG rather than FMG may eliminate the noted negative effects of prelay FMG inoculations on EP and the liver and reproductive organs of layers.

The treatments applied in this study did not affect any of the parameters determined except for liver moisture content. Although the relative weights of the ovary, oviduct, small intestine, and liver were not affected by treatment, the treatment means for relative ovary, oviduct, and small intestine weight are provided in Table 1 and the treatment means for relative liver weight are provided in Table 2 for observation. Furthermore, although FLHS incidence and mature follicle numbers were not affected by treatment, the treatment means for each of these parameters are also subsequently provided for observation. In the control; 6/86MG-10; 6/86MG-10, FMG-22; and 6/86MG-10, FMG-45 treatment groups, mean FLHS incidence levels were 1.44, 1.50, 1.38, and 1.31 (pooled SEM = 0.171), respectively, and mean mature follicle numbers were 4.13, 4.25, 3.75, and 3.92 (pooled SEM = 0.188), respectively.

Knowing the ability of MG to colonize the liver (Sahu and Olson, 1976) and invade cells (Winner et al., 2000), MG would be expected to alter liver lipid metabolism with associated effects on liver weight, histology, and lipid and moisture contents. A significant treatment effect ($P \leq 0.004$) was found for liver moisture concentration, but not for liver weight or lipid concentration in the current study (Table 2). Liver moisture concentration was significantly higher in the 6/85MG-10 and 6/85MG-10, FMG-45 treatment groups in comparison with the 6/85MG-10, FMG-22 and control treatment groups. Peebles et al. (1999) have reported that lipid uptake and storage in the avian liver has an inverse relationship with liver moisture content. Therefore, opposite trends in liver moisture and lipid concentrations would also be expected; however, this was not observed. This suggests that a prelay inoculation of 6/85MG alone or in conjunction with an FMG inoculation overlay at 45 wk can increase liver moisture without affecting liver lipid levels in layers. The observed treatment effects on liver moisture were not associated with those for yolk moisture observed by Viscione et al. (2008a) or with those for plasma protein observed by Peebles et al. (2008). The functional significance of the treatment effects on liver moisture concentration is not understood; nevertheless, it is important to note that the treatment effects on plasma protein and on yolk and liver moisture had no subsequent impact on layer performance, as demonstrated by Viscione et al. (2008b).

In conclusion, these current results in conjunction with those from previous companion studies establish that prelay 6/85MG inoculations may be a suitable substitute for prelay FMG inoculations. The combined results also suggest that the use of FMG inoculation overlays during lay subsequent to prelay 6/85MG vaccinations may provide continual protection against field strain MG infections without eliciting any subsequent

Table 2. Percentages of liver weight (LW) and liver moisture (LM) and lipid (LL) contents in control, 6/85MG at 10 wk (6/85MG-10), 6/85MG at 10 wk and 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 at 58 wk of age[1]

Treatment	LW	LM	LL
		(%)	
Control	1.80	42.5[b]	6.34
6/85MG-10	1.74	47.1[a]	7.17
6/85MG-10, FMG-22	1.87	40.6[b]	6.11
6/85MG-10, FMG-45	1.79	46.7[a]	7.35
Pooled SEM	0.17	10.2	1.31

[a,b]Means within a column (parameter) with no common superscript differ significantly ($P \leq 0.05$).

[1]In each of 2 trials, 4 replicate isolation units, with 2 birds sampled from each unit, were used for calculation of treatment means.

suppressive effects on performance or the digestive and reproductive organs of commercial layers.

REFERENCES

Bligh, E. G., and W. J. Dryer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917.

Branton, S. L., S. M. D. Bearson, B. Bearson, B. D. Lott, W. R. Maslin, S. D. Collier, G. T. Pharr, and D. L. Boykin. 2002. The effects of 6/85 live *Mycoplasma gallisepticum* vaccine in commercial layer hens over a 43-week laying cycle on egg production, selected egg quality parameters, and egg size distribution when challenged before beginning of lay. Avian Dis. 46:423–428.

Burnham, M. R., S. L. Branton, E. D. Peebles, M. S. Jones, P. D. Gerard, and W. R. Maslin. 2002a. Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on digestive and reproductive organ characteristics of commercial egg laying hens. Poult. Sci. 81:1884–1891.

Burnham, M. R., S. L. Branton, E. D. Peebles, B. D. Lott, and P. D. Gerard. 2002b. Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on performance and egg characteristics of commercial egg laying hens. Poult. Sci. 81:1478–1485.

Carpenter, T. E., E. T. Mallinson, K. F. Miller, R. F. Gentry, and L. D. Schwartz. 1981. Vaccination with F-strain *Mycoplasma gallisepticum* to reduce production losses in layer chickens. Avian Dis. 25:404–409.

Frey, M. C., R. P. Hanson, and D. P. Anderson. 1968. A medium for the isolation of avian Mycoplasma. Am. J. Vet. Res. 29:2164–2171.

Kleven, S. H. 1998. Mycoplasmas in the etiology of multifactorial respiratory disease. Poult. Sci. 77:1146–1149.

Kleven, S. H., M. I. Khan, and R. Yamamoto. 1990. Fingerprinting of *Mycoplasma gallisepticum* strains isolated from multiple-age layers vaccinated with live F-strain. Avian Dis. 34:984–990.

Latour, M. A., E. D. Peebles, S. M. Doyle, T. M. Smith, and C. R. Boyle. 1998. Broiler breeder age and dietary fat influence the yolk fatty acid profiles of fresh eggs and newly hatched chicks. Poult. Sci. 77:47–53.

Levisohn, S., and S. H. Kleven. 1981. Vaccination of chickens with nonpathogenic *Mycoplasma gallisepticum* as a means for displacement of pathogenic strains. Isr. J. Med. Sci. 17:669–673.

Mohammed, H. O., T. E. Carpenter, and R. Yamamoto. 1987. Evaluation of factors associated with infection of commercial layers with *Mycoplasma gallisepticum* and *M. synoviae.* Avian Dis. 31:470–476.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Peebles, E. D., L. Li, S. Miller, T. Pansky, S. Whitmarsh, M. A. Latour, and P. D. Gerard. 1999. Embryo and yolk compositional relationships in broiler hatching eggs during incubation. Poult. Sci. 78:1435–1442.

Peebles, E. D., K. A. Viscione, S. L. Branton, A. M. Vance, P. D. Gerard, and S. K. Whitmarsh. 2008. Effects of prelay 6/85-strain *Mycoplasma gallisepticum* inoculation alone or in conjunction with the inoculation of F-strain *M. gallisepticum* during lay on the blood characteristics of commercial egg-laying hens. Poult. Sci. 87:2000–2004.

Sahu, S. P., and N. O. Olson. 1976. Use of the agar-gel precipitin test to evaluate broiler breeder and commercial layer flocks for *Mycoplasma gallisepticum* infection. Avian Dis. 20:563–573.

SAS Institute. 2003. SAS Proprietary Software Release 9.1. SAS Inst. Inc., Cary, NC.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill, New York, NY.

USDA-Animal and Plant Health Inspection Service-Veterinary Services. 2003. National poultry improvement plan and auxiliary provisions. Fed. Regist. 68:28169–28175.

Viscione, K. A., S. L. Branton, P. D. Gerard, S. K. Whitmarsh, and E. D. Peebles. 2008a. Effects of a prelay 6/85-strain *Mycoplasma gallisepticum* inoculation alone or in conjunction with subsequent F-strain *M. gallisepticum* inoculations during lay on the internal egg characteristics of commercial egg-laying hens. Poult. Sci. 87:1120–1124.

Viscione, K. A., S. L. Branton, A. M. Vance, P. D. Gerard, S. K. Whitmarsh, and E. D. Peebles. 2008b. Effects of 6/85-strain *Mycoplasma gallisepticum* inoculation alone at ten weeks of age or in conjunction with F-strain *Mycoplasma gallisepticum* inoculation overlays at twenty-two or forty-five weeks of age on the performance of commercial egg-laying hens. Poult. Sci. 87:588–593.

Winner, F., R. Rosengarten, and C. Citti. 2000. In vitro cell invasion of *Mycoplasma gallisepticum.* Infect. Immun. 68:4238–4244.