

Effects of time-specific F-strain *Mycoplasma gallisepticum* inoculation overlays on prelay ts-11-strain *M. gallisepticum* vaccination on digestive and reproductive organ characteristics of commercial egg-laying hens^{1,2}

A. M. Vance,* S. L. Branton,† S. D. Collier,† P. D. Gerard,‡ and E. D. Peebles*³

*Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762; and †Poultry Research Unit, Agricultural Research Service, USDA, Mississippi State University, Mississippi State, MS 39762; and ‡Department of Applied Economics and Statistics, Clemson University, Clemson, SC 29634

ABSTRACT Two trials were conducted to determine the effects of a prelay ts-11-strain *Mycoplasma gallisepticum* (ts-11MG) vaccination alone or in conjunction with F-strain *M. gallisepticum* (FMG) inoculation overlays at 2 different age periods during lay on the digestive and reproductive organ characteristics of commercial egg-laying hens. In each trial, the following 4 treatments were utilized: sham vaccination at 10 wk of age, ts-11MG vaccination at 10 wk of age, ts-11MG at 10 wk of age overlaid by FMG inoculation at 22 wk of age, and ts-11MG at 10 wk of age overlaid by FMG at 45 wk of age. Necropsies were performed at the end of both trials (58 wk of age), using 2 birds from each of 4 replicate units per treatment, to observe treatment effects on the following parameters: liver weight, liver lipid and moisture concentrations, incidence of fatty

liver hemorrhagic syndrome, ovary weight, number of mature ovarian follicles, and the total and segmental weights, lengths, and histologies of the oviduct and small intestine. Treatments affected only vaginal length as a percentage of total oviduct length. Vaginas were relatively longer in hens that had only been vaccinated with ts-11MG at 10 wk in comparison to all the other treatment groups, including controls. Except for relative vaginal length, the digestive and reproductive organs of layers were not influenced by the ts-11MG and FMG treatment regimens imposed in this study. These results confirm that when coupled with FMG inoculations during lay, prelay ts-11MG vaccinations may be a practical substitute for prelay FMG inoculations for providing continual protection against field-strain *M. gallisepticum* infections in layers.

Key words: layer, liver, oviduct, small intestine, ts-11-strain *Mycoplasma gallisepticum*

2009 Poultry Science 88:980–983
doi:10.3382/ps.2008-00545

INTRODUCTION

Vaccination programs are presently being used to control outbreaks of the more virulent strains of *Mycoplasma gallisepticum* (MG) and prevent subsequent egg production (EP) losses on commercial egg layer facilities maintaining multiage flocks. The F-strain MG (FMG) has long been licensed for use in the United States (Branton et al., 1999) and has proven to protect layers from wild-strain MG infections, but is itself mildly pathogenic (Levisohn and Kleven, 1981). Burnham et al. (2002b) showed that the inoculation of lay-

ers with FMG at 12 wk of age delayed onset of lay and decreased total EP. More recently, ts-11-strain MG (ts-11MG), an apathogenic strain, has also been licensed for use in the United States. This vaccine shows virtually no bird-to-bird transmission but has not been proven to displace wild-type MG (Kleven et al., 1988) or to confer continued protection throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987). Like ts-11MG, the 6/85-strain of MG exhibits minimal virulence, and its use as a prelay vaccine in conjunction with FMG inoculations during lay may lessen the effect of a prelay FMG inoculation while providing continual protection against field-strain MG infections during the pullet period and throughout lay (Branton et al., 2002; Viscione et al., 2008).

In previous companion research using the same treatment regimens and birds as in this study, it was reported that prelay ts-11MG vaccination alone or in conjunction with FMG inoculation during lay did not influence layer EP (Vance et al., 2008a), and except for an isolated treatment effect on serum calcium, the blood char-

©2009 Poultry Science Association Inc.

Received December 14, 2008.

Accepted January 8, 2009.

¹This is journal no. J-11444 from the Mississippi Agricultural and Forestry Experiment station supported by MIS-321010.

²Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.

³Corresponding author: dpeebles@poultry.msstate.edu

acteristics of the layers were not affected (Peebles et al., 2009). However, eggshell pimpling incidence (Vance et al., 2008a), along with various egg yolk and albumen characteristics, were influenced by some of the treatment regimens (Vance et al., 2008a,b). Nevertheless, no information has been published concerning the possible effects of these treatment regimens on the digestive and reproductive organ characteristics of layers. Therefore, the objective of this study was to determine the effects of a prelay (10 wk of age) ts-11MG vaccination alone or in conjunction with FMG inoculations during lay (22 or 45 wk of age) on the digestive and reproductive organ characteristics of commercial laying hens.

MATERIALS AND METHODS

Bird Management

Two trials, conducted under an approved USDA Animal Care and Use protocol, were performed using Hy-Line W-36 pullets that were obtained at 1 d of age from a commercial source that was 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 into each of 16 negative-pressure fiberglass biological isolation units, with 4 units assigned to each of 4 treatment groups. Hen numbers were reduced to 10 per unit at point of lay (22 wk of age) for the duration of each trial (160 total birds per trial). The management and testing of layers for the presence of MG were as described by Vance et al. (2008a). Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. Analyses and ingredient percentages of the diets were as described by Burnham et al. (2002b).

Treatments

Four experimental treatment groups were used. Each treatment group consisted of 4 isolation units containing 10 birds each for a total of 40 birds per treatment group. Treatment 1 (control) received no MG inoculation but was sham-inoculated via eyedrop in the right eye with sterile Frey's media (Frey et al., 1968) at 10 wk of age. Treatment 2 contained birds that were eyedrop-vaccinated in the right eye with ts-11MG at 10 wk of age (ts-11/10). Birds belonging to treatment 3 received ts-11MG via eyedrop in the right eye at 10 wk of age followed by a 22-wk overlay vaccination via eyedrop in the left eye with FMG (ts-11/10-F/22). Treatment 4 consisted of birds given ts-11MG at 10 wk of age via eyedrop in the right eye followed by a 45-wk overlay vaccination of FMG via eyedrop in the left eye (ts-11/10-F/45).

Data Collection

At the end of each trial (wk 56), 2 birds from each replicate unit were killed by cervical dislocation, their organs removed, and the following parameters determined: liver weight, liver moisture and lipid contents, ovary weight, mature ovarian follicle number (those that are ≥ 12 mm in diameter), total weights and lengths of the oviduct and small intestine, and segmental weights and lengths of the oviduct (infundibulum, magnum, isthmus, and vagina) and small intestine (duodenum, jejunum, and ileum). Liver and ovary weights, 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 follicles in an ovary was assigned a category from 0 to 6, where 0 indicated the absence of mature follicles and where 6 was the number of maximum follicles recorded. The percentage of birds in each unit possessing 0, 1, 2, 3, 4, 5, or 6 follicles was calculated. Livers were examined for incidence of fatty liver hemorrhagic syndrome (FLHS) by the same individual observer for all birds in each trial. Two categories were used for classification of FLHS incidence. The FLHS incidence categories were normal and those exhibiting FLHS to any degree. Birds with normal livers or those exhibiting FLHS were calculated as percentages of the total number of birds in each unit.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. The data of both trials were pooled and analyzed together. Trial was considered as a random effect. All data were subjected to 1-way ANOVA. Individual sample data within each of these 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 described previously in the companion article by Vance et al. (2008a), 5-wk-old pullets tested negative for both MG and MS. Throughout the study, control birds remained MG-free and systemic infections were confirmed in MG-treated birds, whether or not they were treated with ts-11MG alone or in combination with FMG. Furthermore, there was no significant difference in mortality between MG-free and MG-inoculated birds in either trial.

Mycoplasma gallisepticum is capable of colonizing the liver and reproductive organs of birds (Carlson and Howell, 1967; Sahu and Olson, 1976). Peebles et al. (2007) showed that the period [pullet (12 wk) vs. hen (22 wk)] at which layers are inoculated (sham or FMG) can affect their reproductive organ characteristics and, more specifically, that the period at which an FMG inoculation is given can affect small intestinal structure. Furthermore, Branton et al. (2003) demonstrated increased incidences of FLHS in commercial layers in response to a *Mycoplasma gallinarum* infection. It has also been previously reported by Burnham et al. (2002b) that the production of undersized eggs was shifted later into lay, onset of EP was delayed by 1 wk, and that total EP and weekly EP (after 42 wk of age) were reduced in layers inoculated with the FMG at 12 wk of age. Burnham et al. (2002a) later reported that hens inoculated with FMG at 12 wk had fewer mature ovarian follicles; decreased magnal, isthmal, and vaginal proportions of the reproductive tract; and increased incidences of FLHS at trial termination (60 wk) compared with FMG-free hens. The suggestion was made by Burnham et al. (2002a) that alterations in the performance and egg characteristics of the layers examined were related to mutual functional disturbances in the liver, ovary, and oviduct.

However, in a study by Branton et al. (2000), in which the ts-11MG vaccine was administered at 10 wk of age, no significant effects were observed on subsequent mortality, EP, egg size, incidences of egg blood and meat spots, or Haugh unit scores. This suggested that the ovary and various segments of the oviduct were not compromised by the ts-11MG vaccine. Furthermore, gross and histological changes were not observed upon necropsy. Nevertheless, inoculation of FMG at 45 wk subsequent to vaccination of ts-11MG at 10 wk has been demonstrated to increase postpeak incidences of eggshell pimpling and egg blood spots (Vance et al., 2008a), and ts-11MG at 10 wk alone has decreased postpeak Haugh unit scores (Vance et al., 2008b). In addition, yolk lipid percentage increased at 32 wk and relative albumen weight decreased during postpeak lay in eggs from hens treated with ts-11MG at 10 wk before FMG at 22 wk (Vance et al., 2008b). Vaccination of ts-11MG at 10 wk overlaid by FMG inoculation at 22 wk also elevated serum calcium concentrations above those of layers vaccinated with ts-11MG alone, and both of the above treatments increased serum calcium levels over those of sham-vaccinated controls (Peebles et al., 2009). However, with the exception of these effects, prelay (10 wk of age) ts-11MG vaccination alone or in conjunction with subsequent overlay inoculations of FMG during lay (22 or 45 wk of age) did not influence the performance (including weekly and total EP; Vance et al., 2008a), internal egg and eggshell characteristics (Vance et al., 2008b), or blood characteristics (Peebles et al., 2009) of commercial layers.

In the present study, no significant treatment effects were observed for any of the digestive and reproduc-

Table 1. Vagina weight as a percentage of BW (VAGBW), vagina weight as a percentage of oviduct weight (VAGOW), and vagina length as a percentage of oviduct length (VAGOL) in control, ts-11-strain *Mycoplasma gallisepticum* at 10 wk (ts-11MG-10), ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 22 wk (ts-11MG-10, FMG-22), and ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 45 wk (ts-11MG-10, FMG-45) treatment groups at 58 wk of age¹

Treatment	VAGBW	VAGOW	VAGOL
	(%)		
Control	0.226	5.45	5.49 ^b
ts-11MG-10	0.210	5.53	7.30 ^a
ts-11MG-10, FMG-22	0.177	5.73	5.64 ^b
ts-11MG-10, FMG-45	0.215	4.98	5.36 ^b
Pooled SEM	0.062	2.19	1.42

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

¹Within each column (parameter), 2 birds were sampled from each of 8 replicate isolation units for the calculation of treatment means.

tive organ characteristics examined, except for vaginal length as a percentage of total oviduct length. Vaginas were relatively longer in hens that had only been vaccinated with ts-11MG at 10 wk in comparison to all the other treatment groups, including controls (Table 1). The treatment regimens used in this study, which were the same as those used by Vance et al. (2008a,b) and Peebles et al. (2009), also did not have a detrimental effect on the liver as evidenced by the absence of changes in FLHS incidence, liver weight, and liver lipid and moisture contents. Although only relative vaginal length was affected by treatment in this study, the following parameters are also displayed in tabular form for observation: percentages of liver weight, and of liver moisture and lipid contents (Table 2); percentages of ovary, oviduct, and small intestine weight (Table 3); and vagina weight as a percentage of BW and of total oviduct weight (Table 1).

In conclusion, the use of a prelay ts-11MG vaccination at 10 wk alone may result in increased relative vagina length in the oviduct. Furthermore, upon consideration of the current results and those from earlier studies, it

Table 2. Liver weight as a percentage of BW (LW) and liver moisture (LM) and liver lipid (LL) concentrations in control, ts-11-strain *Mycoplasma gallisepticum* at 10 wk (ts-11MG-10), ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 22 wk (ts-11MG-10, FMG-22), and ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 45 wk (ts-11MG-10, FMG-45) treatment groups at 58 wk of age¹

Treatment	LW	LM	LL
	(%)		
Control	1.68	48.1	3.61
ts-11MG-10	1.59	48.1	4.54
ts-11MG-10, FMG-22	1.74	47.7	3.72
ts-11MG-10, FMG-45	1.69	47.3	3.83
Pooled SEM	0.08	22.1	1.06

¹Within each column (parameter), 2 birds were sampled from each of 8 replicate isolation units for the calculation of treatment means.

Table 3. Ovary weight (OVAW), oviduct weight (OVIW), and small intestine weight (SIW) as percentages of BW in control, ts-11-strain *Mycoplasma gallisepticum* at 10 wk (ts-11MG-10), ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 22 wk (ts-11MG-10, FMG-22), and ts-11-strain *M. gallisepticum* at 10 wk and F-strain *M. gallisepticum* at 45 wk (ts-11MG-10, FMG-45) treatment groups at 58 wk of age¹

Treatment	OVAW	OVIW	SIW
	(%)		
Control	2.90	4.19	1.52
ts-11MG-10	2.59	4.12	1.48
ts-11MG-10, FMG-22	2.60	3.65	1.63
ts-11MG-10, FMG-45	2.92	4.31	1.48
Pooled SEM	0.26	0.41	0.08

¹Within each column (parameter), 2 birds were sampled from each of 8 replicate isolation units for the calculation of treatment means.

is suggested that despite the effects of various ts-11MG vaccine and FMG inoculation treatment combinations on relative vaginal length and on some egg (Vance et al., 2008a,b) and blood (Peebles et al., 2009) characteristics in layers, these treatment regimens may overcome some of the adverse effects of an individual prelay FMG inoculation on performance, as noted by Burnham et al. (2002b), while eliminating the threat of field-strain MG infections.

ACKNOWLEDGMENTS

This work was funded by a grant by the USDA. We express appreciation to fellow workers of the USDA Poultry Research Unit and Sharon Womack of the Mississippi State University Poultry Science Department.

REFERENCES

- 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.
- Branton, S. L., S. M. D. Bearson, B. L. Bearson, W. R. Maslin, S. D. Collier, J. D. Evans, D. M. Miles, and G. T. Pharr. 2003. *Mycoplasma gallinarum* infection in commercial layers and onset of fatty liver hemorrhagic syndrome. *Avian Dis.* 47:458–462.
- Branton, S. L., B. D. Lott, J. D. May, W. R. Maslin, G. T. Pharr, S. D. Bearson, S. D. Collier, and D. L. Boykin. 2000. The effects of ts-11 strain *Mycoplasma gallisepticum* vaccination in commercial layers on egg production and selected egg quality parameters. *Avian Dis.* 44:618–623.
- Branton, S. L., B. D. Lott, J. D. May, W. R. Maslin, G. T. Pharr, J. E. Brown, and D. L. Boykin. 1999. The effects of F-strain *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and the dual infection in commercial layer hens over a 44-week laying cycle when challenged before beginning of lay. II. Egg size distribution. *Avian Dis.* 43:326–330.
- 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.
- Carlson, H. G., and J. Howell. 1967. Serological and cultural studies of chicken breeding flocks and their progeny for *Mycoplasma*. *Avian Dis.* 11:24–28.
- 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., H. H. Frank, and K. S. Turner. 1988. Pen trial studies on use of live vaccines to displace virulent *Mycoplasma gallisepticum* in chickens. *Avian Dis.* 52:300–306.
- 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. Economic impact of *Mycoplasma gallisepticum* and *M. synoviae* in commercial layer flocks. *Avian Dis.* 31:477–482.
- NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Peebles, E. D., S. L. Branton, M. R. Burnham, S. K. Whitmarsh, and P. D. Gerard. 2007. Effects of supplemental dietary phytase and 25-hydroxycholecalciferol on the digestive and reproductive organ characteristics of commercial layers inoculated before or at the onset of lay with the F-strain of *Mycoplasma gallisepticum*. *Poult. Sci.* 86:1805–1809.
- Peebles, E. D., A. M. Vance, S. L. Branton, S. D. Collier, and P. D. Gerard. 2009. 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. *Poult. Sci.* 88:911–916.
- 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 Institute 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.
- Vance, A. M., S. L. Branton, S. D. Collier, P. D. Gerard, and E. D. Peebles. 2008a. Effects of time-specific F-strain *Mycoplasma gallisepticum* inoculation overlays on prelay ts11-strain *Mycoplasma gallisepticum* inoculation on performance characteristics of commercial laying hens. *Poult. Sci.* 87:655–660.
- Vance, A. M., S. L. Branton, S. D. Collier, P. D. Gerard, and E. D. Peebles. 2008b. Effects of prelay ts11-strain *Mycoplasma gallisepticum* inoculation and time-specific F-strain *M. gallisepticum* inoculation overlays on internal egg and eggshell characteristics of commercial laying hens. *Poult. Sci.* 87:1358–1363.
- Viscione, K. A., S. L. Branton, A. M. Vance, P. D. Gerard, S. K. Whitmarsh, and E. D. Peebles. 2008. 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.
- Yoder, H. W., Jr. 1978. *Mycoplasma gallisepticum* infections. Pages 236–250 in *Diseases of Poultry*. 7th ed. M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder Jr., ed. Iowa State University Press, Ames.
- Yoder, H. W., Jr. 1991. Mycoplasmosis. Pages 198–212 in *Diseases of Poultry*. 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder Jr., ed. Iowa State University Press, Ames.