Effects of different vaccine combinations against *Mycoplasma gallisepticum* on the internal egg and eggshell characteristics of commercial layer chickens\(^1,2,3\)

R. Jacob, * S. L. Branton, † J. D. Evans, ‡ S. A. Leigh, † and E. D. Peebles* \(^4\)

*Department of Poultry Science, Mississippi State University, Mississippi State 39762; and † USDA-Agricultural Research Service, Poultry Research Unit, Mississippi State, MS 39762

**ABSTRACT** Live F-strain *Mycoplasma gallisepticum* (FMG) vaccines are presently being used to help control field-strain MG outbreaks. However, they may exert some adverse effects on egg production. Live strains of MG of lesser virulence as well as killed vaccines have little or no effect on egg production, but afford lower levels of protection. This has led to research investigating their use in combination with a subsequent overlay vaccination of FMG given later in the production cycle. In the present study, 2 trials were conducted to investigate the effects of prelay vaccinations of live and killed MG vaccines or their combination, in conjunction with an FMG vaccine overlay after peak production, on the egg characteristics of commercial layers. The following vaccination treatments were administered at 10 wk of age (woa): 1) unvaccinated (Control), 2) MG–Bacterin (MGBac) vaccine, 3) ts-11 strain MG (ts11MG) vaccine, and 4) MGBac and ts11MG combination (MGBac + ts11MG). At 45 woa, half of the birds were overlaid with an FMG vaccine. In each trial, internal egg and eggshell parameters including egg weight (EW), Haugh unit score (HU), eggshell breaking strength (EBS), percentage yolk weight (PYW), percentage albumen weight (PAW), percentage eggshell weight (PSW), eggshell weight per unit surface area (SWUSA), percentage yolk moisture (PYM), and percent total lipids (PYL) were determined at various time periods between 21 and 52 woa. At 28 woa, SWUSA was lower in the ts11MG and MGBac + ts11MG groups compared to the Control group. Conversely, at 43 woa, SWUSA was higher in the ts11MG than in the MGBac group. Between 23 and 43 woa, PYL was higher in the MGBac and ts11MG groups in comparison to the Control group. In conclusion, vaccination with MGBac alone or in combination with ts11MG at 10 woa with or without an FMG vaccine overlay at 45 woa does not adversely affect the internal egg or eggshell quality of commercial layers throughout lay.

**Key words:** bacterin, commercial layer, egg quality, *Mycoplasma gallisepticum*, vaccine

**INTRODUCTION**

Chronic respiratory disease in chickens, caused by *Mycoplasma gallisepticum* (MG), continues to be a costly problem for commercial table egg producers maintaining multi-age layer houses (Ley, 2008). Losses associated with reduced egg production (EP) and egg size are recognized as major consequences of MG infection in layers (Branton et al., 1999). Nevertheless, in addition to increased medication costs, losses due to MG can also result from a decrease in feed efficiency (Mohammed et al., 1987; Ley, 2008). Efforts at reducing MG-related losses have resulted in the vaccination of birds with the F-strain of MG (FMG) as an intermediary step towards eradication. When administered at 12 or 22 wk of age (woa), FMG has been shown to reduce the EP of layers (Peebles et al., 2008). Despite its effects on EP, it was also reported by Peebles et al. (2008) that egg weight, eggshell quality, relative weights of the yolk and albumen, and yolk moisture and lipid concentrations were not affected by the inoculation of FMG at either 12 or 22 woa. Conversely, using the same vaccination regimen, Park et al. (2009a) reported that although it had no effect on eggshell quality, egg weight was increased by 1.7 g throughout lay, and Park et al. (2009b) further reported that total yolk lipid concentration in eggs laid at 24 woa was reduced by 3.7%. However, despite a
potential increase of 8.7 eggs/hen housed over a 45-wk laying period in MG-free versus FMG-vaccinated hens (Carpenter et al., 1981), layer producers prefer vaccination as a potential way to prevent wild-type or field-strain MG infections.

Although eradication remains the ultimate goal in MG control, investigations are needed to identify alternatives to the currently available vaccination regimens. Alternative regimens may include the individual and combined use of killed and live MG vaccine strains of low virulence. Vaccines derived from avirulent MG strains such as ts-11 strain MG (ts11MG), and killed vaccines such as MG–Bacterins (MGBac) have also been widely used in layer chickens. Even though these vaccines do not show any apparent bird-to-bird transmission and do not result in a decrease in EP, as may occur in response to an FMG vaccination, they have not been proven to displace previously colonized wild-type MG (Kleven, 1998).

Nevertheless, ts11MG offers protection against airsacculitis associated with virulent MG challenges (Whithear et al., 1990; Evans and Hafez, 1992; Abd-El-Motelib and Kleven, 1993), and MGBac has been shown to provide protection against virulent MG challenges in chickens up to 7 wk postvaccination (Hildebrand et al., 1983; Hildebrand, 1985). Branton et al. (2000) determined that a ts11MG vaccination at 10 wo had no effect on egg size, eggshell breaking strength (EBS), egg Haugh unit score (HU), or incidences of eggshell pimpling and egg blood and meat spots, whereas Vance et al. (2008b) showed increases in the incidences of eggshell pimpling and internal blood spots of eggs laid at 56 wo in response to inoculations of ts11MG at 10 wo followed by an FMG inoculation at 45 woa. Also, Vance et al. (2008a) reported that ts11MG vaccination at 10 wo alone increased percentage yolk lipid (PYL) by 2.4% at 32 woa, and in conjunction with a subsequent vaccination of FMG at 22 woa, increased PYL by 2.6% at 32 woa followed by a decrease in percentage albumen weight (PAW). However, when the ts11MG vaccine was administered alone at 10 wo in conjunction with a subsequent FMG vaccination overlay at 45 woa, it had no effect on PYL at 47 or 56 woa. No studies have been conducted to evaluate the potential effects of an MGBac vaccine on the egg and eggshell quality of layers.

The lower level of protection afforded by low virulence MG strain vaccines makes it more advisable to administer FMG as an overlay vaccine later in the production cycle. The effects of individual or combined prelay (10 woa) MGBac and ts11MG vaccinations with or without a subsequent FMG vaccination overlay after peak EP (45 woa) on production parameters of commercial layers were examined in a companion article by Jacob et al. (2014). It was shown that individual use of ts11MG, MGBac, or their combination at 10 wo did not significantly affect layer performance. However, it was also suggested that while providing continual protection against field-strain MG infection when used before an FMG vaccination given during lay, a prelay vaccination with ts11MG, but not MGBac, may not only lack any suppressive effects on EP, but may further reduce the negative impacts of a subsequent FMG overlay on hen performance. Possible changes in the internal egg and eggshell characteristics of hens subjected to vaccination regimens involving MGBac like the ones used by Jacob et al. (2014) have not been previously examined. Therefore, the objective of this study was to examine the effect of the individual or combined use of prelay (10 woa) vaccination with ts11MG and MGBac with or without an associated FMG vaccination overlay during postpeak EP (45 woa) on the internal egg and eggshell quality of laying hens.

MATERIALS AND METHODS

Pullet Housing and Management

In each of 2 trials, Hy-Line W-36 commercial laying chicks were obtained from an MG- and Mycoplasma synoviae-free commercial source on the day of hatch. The chicks were placed in a conventional house on clean, dry pine shavings until 9 woa. Initial stocking density was 0.034 m²/bird. An artificial lighting schedule was followed daily in the conventional house with a 13L:11D daily light cycle. In order to provide 35.5 lux light intensity at bird level, one 75-watt incandescent light bulb was used for each 8.4 cm² floor space.

Layer Housing and Management

In each trial, the experimental birds were transferred at 9 woa into an environmentally controlled disease isolation facility with temperature-controlled negative-pressure fiberglass biological isolation units. Eleven pullets were randomly selected and placed into each of 16 isolation units. The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992), and were maintained under the same conditions described by Evans et al. (2012). Four birds/unit were also bled and serum was used for the detection of MG infection by the serum plate agglutination test at the time of placement in the isolation units. Choanal cleft swabs were also collected from all the birds at 9, 45, and 52 woa to assess for the presence or absence of MG infection according to treatment by PCR-based DNA detection techniques (Kleven, 2008). Samples collected at 45 woa were obtained prior to the overlay of FMG. The birds used in the current study were the exact same as those used in the study by Jacob et al. (2014). The materials and methods of MG screening were described by Jacob et al. (2014).

When the total daily EP of the birds reached 10%, one bird in each unit was randomly selected and removed from the study to reduce the total 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 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. All procedures were approved by the USDA–Agricultural Research Service Institutional Animal Care and Use Committee. Beginning at 18 woa, the artificial lighting schedule was increased by 15 min/wk from that which was initiated at 9 woa, until a cycle of 16 h 15 min light and 7 h 45 min dark was achieved as described previously (Branton et al., 2002). Layers were maintained on that schedule throughout the remainder of the study.

Pullet and Layer Diets

Throughout the pullet and layer periods of each trial, chickens had ad libitum access to feed and water. Birds received standard diets, previously described by Burnham et al. (2002) that met or exceeded NRC (1994) recommendations. In each trial, diets were formulated in accordance with the starter (0 to 6 woa), grower (6 to 12 woa), developer (12 to 18 woa), prelay (18 woa to onset of lay), and lay (onset of lay to 52 woa) phases of the birds’ production cycle.

Treatments

In each trial, 4 isolation units containing 10 birds each were assigned to one of 4 treatment groups (40 total birds per treatment group) at 10 woa. The following vaccine treatments were administered at 10 woa. Treatment 1 (Control) was unvaccinated. Treatment 2 (MGBac) consisted of birds that were vaccinated with MGBac (MG-Bac, Fort Dodge Animal Health, Overland Park, KS) via intramuscular (breast) injection. Birds belonging to treatment 3 (ts11MG) were eye-drop-vaccinated in the left eye with ts11MG (Mycoplasma Gallisepticum Vaccine, Merial Select, Gainesville, GA). In treatment group 4 [MGBac and ts11MG combination (MGBac + ts11MG)], birds were vaccinated with a combination of MGBac and ts11MG.

At 45 woa, the birds in 2 replicate units in each of the treatment groups were overlay vaccinated with a laboratory stock of FMG (99th passage above the unknown passage level), thereby increasing the number of the treatments to 8. A 24-h broth culture of the FMG was produced in Frey’s broth medium (Frey et al., 1968) and 20 μL of the overnight culture was applied via eye-drop vaccination. Titers of the ts11MG vaccine in trials 1 and 2 were 2.45 × 10^7 and 8.65 × 10^6 cfu/mL, respectively. Titers of the FMG vaccine administered at 45 woa in trials 1 and 2 were 6.45 × 10^7 and 5.48 × 10^7 cfu/mL, respectively. The husbandry and handling procedures employed minimized the risk of cross-contamination.

Data Collection

All data collected from 21 to 45 woa were designated as belonging to age interval I, and all data collected from 46 to 52 woa were designated as belonging to age interval II. In both trials, eggs were collected on 2 consecutive d each wk from 21 to 52 woa for determination of HU, and from 22 to 52 woa for determination of EBS. Determinations of HU and EBS were made on the same d that the eggs were collected and on the same eggs that were produced over the 2-d collection period. Approximately 6 to 10 eggs per replicate pen were used on each d of collection. For determination of egg weight (EW), percentage yolk weight (PYW), PAW, percentage eggshell weight (PSW), eggshell weight per unit of surface area (SWUSA), percentage yolk moisture (PYM), and PYL, a total of 10 eggs per replicate pen were collected at 23, 28, 33, 38, 43, 47, and 52 woa in both trials. If less than 10 eggs were collected from a replicate pen on a given d, more eggs were collected on the following d of that wk so that the total number of eggs collected equaled 10. Except for PYM and PYL, all other measurements were made on the same d that the eggs were collected.

Methods of Internal Egg and Eggshell Quality Determinations

A stress-strain measuring instrument (Instron Model No. 5544; Instron Corporation, Norwood, MA), as previously described by Reece and Lott (1976), was used to determine EBS, which was expressed as kilograms breaking force. An egg quality management system (Technical Services and Supplies Limited, York, UK), for the measurement of albumen quality, was used to determine HU. Relative weights of the yolk (PYW), albumen (PAW), and shell (PSW) were expressed as percentages of total EW. Determination of SWUSA, a measure of eggshell thickness, was performed according to the procedure described by Peebles et al. (1992) and was expressed as milligrams per square centimeter. For the determination of PYM, a 2-g yolk sample was dried and its weight recorded as previously described by Peebles et al. (1999). Yolk moisture was calculated as the difference between the initial wet and final dry weight of the sample, and was expressed as a percentage of initial wet sample weight. For the determination of PYL, total lipids were extracted from a 3-g yolk sample using chloroform–methanol (2:1 vol/vol) by the method of Bligh and Dryer (1959). Yolk lipid concentration was expressed as a percentage of initial wet sample weight.

Statistical Analysis

Designating trial as the blocking factor, a generalized randomized complete block design was used. All data collected from 21 to 45 woa were designated as belonging to age interval I, and all data collected from
RESULTS AND DISCUSSION

For both trials, serological screenings and MG-specific PCR analysis indicated that all birds had no exposure to MG prior to treatment. Furthermore, PCR analysis at 45 and 52 woa indicated that MG–DNA was or was not detected in the birds in accordance with their treatment, and that the MG–DNA of the ts11MG and MGBac + ts11MG treatments were similar (Jacob et al., 2014). For sake of brevity and because previous reports have provided age-related data for the parameters investigated, only those data involving significant main or interactive effects due to treatment are provided and discussed. There were no significant main or interactive effects due to bird age or treatment, in either age interval for PSW or PYM. Furthermore, there were only significant age main effects for HU (P ≤ 0.0001), EBS (P ≤ 0.0002), EW (P ≤ 0.0001), PYW (P ≤ 0.001), and PAW (P ≤ 0.02) in age interval I. No treatment effects were observed in age interval I, and no effects due to age or treatment were observed in age interval II for HU, EBS, EW, PYW, or PAW. Previous results from a companion study by Jacob et al. (2014), using these same birds, showed that EP across the entire lay cycle was not affected by vaccination treatment. Total percentage EP across lay for the Control, MGBac, ts11MG, and MGBac + ts11MG vaccination treatment groups was 83.1, 81.6, 82.1, and 81.1%, respectively. Percentage EP across lay for these respective vaccination treatment groups that were later overlaid with the FMG vaccine at 45 woa was 79.5, 81.2, 82.4, and 82.8%, (pooled SEM = 3.47).

Similar to the results of Branton et al. (2000), a ts11MG vaccination at 10 woa did not affect HU throughout the production cycle in the current study. Vance et al. (2008a) also later observed that a ts11MG vaccination at 10 woa together with an FMG overlay vaccination at 45 woa did not affect weekly determinations of HU from 45 throughout 57 woa. Conversely, Branton et al. (1988) observed that HU increased subsequent to an FMG vaccination alone at 45 woa, and Vance et al. (2008a) observed that HU throughout a 45 to 57 woa production period decreased in response to an individual ts11MG vaccination at 10 woa. Although the results of Vance et al. (2008a) suggested that a prelay ts11MG vaccination alone may lower albumen quality during postpeak lay, the current results in addition to those of the other aforementioned studies indicate that individual and combined prelay vaccinations of ts11MG and MGBac alone or in conjunction with a subsequent FMG vaccination overlap at 45 woa do not exert any deleterious effects on albumen quality throughout lay.

The results observed herein for EBS, EW, PYW, PAW, and PYM are in agreement with those reported by Branton et al. (2000) and Vance et al. (2008b,a). Vance et al. (2008b) reported that ts11MG vaccination at 10 woa had no effect on weekly determinations of EBS or EW from 23 to 44 woa, and that ts11MG vaccination at 10 woa alone or in conjunction with a subsequent FMG vaccination overlap at 45 woa had no effect on weekly determinations of EBS or EW from 45 to 57 woa. In addition, Vance et al. (2008a) reported that a ts11MG vaccination at 10 woa did not affect PYW, PAW, PSW, or PYM at 24, 32, or 43 woa, and that a ts11MG vaccination at 10 woa alone or preceding an FMG vaccination at 45 woa, had no effect on these same parameters at 47 or 56 woa. Therefore, our data confirms and supports the previous reports by Branton et al. (2000) and Vance et al. (2008a,b) indicating that prelay vaccination with ts11MG with or without an FMG vaccination during lay has no repercussions on the EBS, EW, PYW, PAW, or PSW of table eggs. Furthermore, the current data uniquely showed that vaccination with MGBac alone or in combination with ts11MG at 10 woa with or without a subsequent overlay vaccination with FMG at 45 woa had no influence on any of the above parameters.

There was a significant age × treatment interaction (P ≤ 0.05) for SWUSA in age interval I (Table 1). There was also a significant main effect due to treatment for PYL in age interval I (P ≤ 0.04) (Table 2). There were no significant main or interactive effects due to bird

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Table 1. Mean eggshell weight per unit of surface area at 23, 28, 33, 38, and 43 wk hen age (interval I) across trials 1 and 2 for unvaccinated control (Control), and for MGBac, ts11MG, and MGBac + ts11MG vaccination treatments administered at 10 wk age.1,2

<table>
<thead>
<tr>
<th>wk age</th>
<th>Control</th>
<th>MGBac</th>
<th>ts11MG</th>
<th>MGBac + ts11MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>78.4</td>
<td>78.6</td>
<td>78.2</td>
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<td>28</td>
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<td>79.2a</td>
<td>79.1b</td>
</tr>
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<td>79.6</td>
<td>80.7</td>
<td>80.6</td>
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<td>81.1</td>
<td>80.4</td>
<td>80.5</td>
</tr>
<tr>
<td>43</td>
<td>79.9a</td>
<td>78.9</td>
<td>81.0a</td>
<td>79.9a</td>
</tr>
</tbody>
</table>

1SEM based on pooled estimate of variance = 0.71.
2Ten eggs from each of 4 replicate units in each of 2 replicate trials were used to calculate treatment means at each wk age. a,b Means within a row with no common superscript differ (P ≤ 0.05).
Table 2. Mean percentage egg yolk lipid concentration in age interval I (across wk 23, 28, 33, 38, and 43) and across trials 1 and 2 for unvaccinated control (Control), and for MGBac, ts11MG, and MGBac + ts11MG vaccination treatments administered at 10 wk age1,2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean PYL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.8</td>
</tr>
<tr>
<td>MGBac</td>
<td>23.9</td>
</tr>
<tr>
<td>ts11MG</td>
<td>23.8</td>
</tr>
<tr>
<td>MGBac + ts11MG</td>
<td>23.0</td>
</tr>
</tbody>
</table>

1SEM based on pooled estimate of variance = 0.67. 
2Ten eggs from each of 4 replicate units at each of 5 time periods and in each of 2 replicate trials were used to calculate treatment means. 

Means with no common superscript differ (P ≤ 0.05).

age for PYL in interval I, and there were no significant effects due to age or treatment for SWUSA or PYL in age interval II. The mean SWUSA of eggs from hens that were 28 wk old was lower in those belonging to the ts11MG and MGBac + ts11MG treatment groups in comparison to those in the Control group. The SWUSA of eggs laid by hens in the MGBac treatment group was intermediate to the other 3 groups. Conversely, at 43 wk old, SWUSA was lower in the MGBac treatment group in comparison to the ts11MG treatment group, with that in the Control and MGBac + ts11MG groups being intermediate (Table 1). Mean PYL was higher in eggs from hens belonging to the MGBac group in comparison to that of eggs from hens in the Control and MGBac + ts11MG groups, and the PYL of eggs laid by hens in the ts11MG treatment group was higher than that of eggs laid by hens in the Control group.

Vance et al. (2008b) reported that ts11MG vaccine administered at 10 wk old had no effect on SWUSA at 20, 24, 32, or 43 wk old, and that ts11MG vaccine administered at 10 wk old alone or in conjunction with an FMG vaccination at 45 wk old had no effect on SWUSA at 46 wk old. Their findings partially disagreed with our observations concerning SWUSA. However, based on comparisons with the Control group, the isolated treatment effect that was observed exclusively at 28 wk suggests only a short-lived effect of a prelay ts11MG vaccination alone or in combination with MGBac on eggshell quality, and specifically on eggshell thickness (SWUSA). Therefore, although MG infection of the oviduct may occur due to its close proximity to the air sacs (Pruthi and Kharole, 1981; Nunoya et al., 1997), the absence of any treatment effects on HU, EBS, PAW, and PSW, and only a sporadic effect on SWUSA, suggests that the treatments applied in this study did not appreciably affect the normal functioning of the oviduct, particularly those regions of the oviduct involved with albumen and shell formation.

*Mycoplasma gallisepticum* organisms have been previously isolated from the liver (Sahu and Olson, 1976) and periovarian region (Fabricant and Levine, 1963) of chickens, and have been shown to be capable of invading cells (Winner et al., 2000). Roberts and McDaniel (1967) have also suggested that MG organisms may be able to cross-infect the ovaries from adjacent colonized air sacs in females. Burnham et al. (2003) have shown that alterations in the EP of commercial layers in response to an FMG infection at 12 wk old are associated with changes in yolk composition, and suggested that a relationship exists between alterations in egg yolk composition and disturbances in the functionalities of the liver and reproductive system. Colonization of MG in cells of the liver and ovary has the potential to alter the PYL of eggs through its possible influence on lipid synthesis in the liver and the subsequent deposition of circulating lipids in the ovarian follicles. Similar to the results of Vance et al. (2008a), ts11MG vaccine administered alone at 10 wk old was further shown to increase PYL across wk 23, 28, 33, 38, and 43 in the present study. In addition, MGBac alone at 10 wk old was shown to induce a response that resembled that produced by ts11MG vaccine. The current results confirm those of Vance et al. (2008a), indicating that ts11MG vaccination at 10 wk old either alone or in conjunction with FMG at 45 wk old has no effect on PYL during postpeak lay, but that the PYL of hens in the earlier period of lay, throughout 43 wk old, may be increased by a prelay vaccination with ts11MG. The results of the present study further suggest that an individual prelay vaccination of MGBac may, like ts11MG vaccination, increase PYL during the same period of lay. However, the lack of effect on PYL in response to the MGBac + ts11MG vaccination treatment was surprising, in view of the significant individual effects of the MGBac and ts11MG vaccines on PYL.

In conclusion, the results of this study confirm that because of an isolated negative effect of an individual ts11MG vaccination at 10 wk old on SWUSA at 28 wk old, the individual vaccination with ts11MG at 10 wk old alone or in conjunction with an overlay vaccination with FMG at 45 wk old does not have an appreciable negative influence on the normal function of the reproductive systems of commercial layer chickens or their subsequent internal egg and eggshell characteristics throughout lay. Furthermore, current results uniquely indicate that a prelay (10 wk) MGBac vaccination alone or in combination with ts11MG administered without or in conjunction with an FMG overlay vaccination during lay (45 wk old) does not by itself influence the internal egg or eggshell characteristics of commercial layers as well as the functionality of their reproductive systems throughout lay. The MGBac results reported in this paper are likewise in agreement with the performance results reported previously by Jacob et al. (2014).

REFERENCES


EFFECTS OF MYCOPLASMA GALLISEPTICUM VACCINATION 917


