Effects of Time-Specific F-Strain *Mycoplasma gallisepticum* Inoculation Overlays on Prelay ts11-Strain *Mycoplasma gallisepticum* Inoculation on Performance Characteristics of Commercial Laying Hens[1,2]

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

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

ABSTRACT *Mycoplasma* bacteria are virtually ubiquitous in layer chicken flocks, and *Mycoplasma gallisepticum* is the species of greatest concern to commercial egg producers. Live *M. gallisepticum* vaccines were initially approved by the USDA for use in commercial layers in 1988 to help control *M. gallisepticum* outbreaks. In the present study, 2 trials were conducted to determine the effects of 2 currently available live *Mycoplasma* vaccines (the ts11- and F-strains) when used together. The following 4 inoculation treatments were used: 1) sham inoculation at 10 wk of age, 2) ts11 at 10 wk, 3) ts11 at 10 wk overlaid by the F-strain at 22 wk, and 4) ts11 at 10 wk overlaid by the F-strain at 45 wk. In each trial, at various ages between 18 and 57 wk of age, hen mortality; BW; egg weight; egg production; eggshell breaking strength; incidences of egg blood spots, egg meat spots, and eggshell pimpling; and eggshell weight per unit of surface area were assessed. The effects of inoculation treatment on egg weight at 27, 37, and 38 wk were inconsistent and variable. Eggshell pimpling and egg blood spot incidences at 56 wk were highest in eggs belonging to the ts11 at 10 wk/F-strain at 45 wk group. Despite increases in pimpling and blood spot incidences very late in production because of the ts11 at 10 wk/F-strain at 45 wk treatment, performance in layers was not adversely affected by a 10-wk ts11 inoculation alone or in conjunction with subsequent overlay inoculations of the F-strain during lay. It is therefore suggested that the 10-wk inoculation of commercial layers with ts11 may reduce the negative impacts of a prelay F-strain inoculation on performance, as reported in earlier studies, while providing protection against subsequent field strain *M. gallisepticum* infections. Furthermore, the ts11- and F-strain *M. gallisepticum* treatment combinations may overcome some of the inadequacies that prelay ts11- or F-strain *M. gallisepticum* vaccines may have when given independently.

Key words: commercial layer, egg production, egg quality, F-strain *Mycoplasma gallisepticum*, ts11-strain *Mycoplasma gallisepticum*

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INTRODUCTION

Mycoplasma gallisepticum, the pathogen responsible for chronic respiratory disease in chickens, continues to be a concern to commercial table egg producers maintaining multiage layer houses. Once a bird is infected with *M. gallisepticum*, it is considered to be chronically infected for life (Brown et al., 1995). Primarily a respiratory pathogen of meat-type chickens and turkeys and a reproductive pathogen of table egg chickens, *M. gallisepticum* causes great economic loss worldwide because of its ability to systemically infect individual birds and the ease at which it is transmitted between birds. Decreased egg production (**EP**) and hatchability, downgrading and condemnation of carcasses, and reduced feed conversion can result from *M. gallisepticum* infections (Yoder and Hofstad, 1964; Domermuth et al., 1967; Patterson, 1994).

Vaccination programs are presently being used to help protect flocks from field strains of *Mycoplasma.* Three live vaccines are commercially available for use in the United States. The first live *M. gallisepticum* vaccine, referred to as the F-strain, was approved for use by the USDA in commercial table egg chickens in 1988 (Branton et al., 1999). The F-strain *M. gallisepticum* vaccine strain is less virulent than many of the field strains of *M. gallisepticum* and has a lower vertical transmission rate, yet it is able to displace the more virulent stains of *M. gallisepticum* (Levisohn and

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[3]Corresponding author: dpeebles@poultry.msstate.edu

Kleven, 1981; Kleven et al., 1990). Continuous use of F-strain *M. gallisepticum* vaccines for replacement flocks in multiage commercial layer facilities has been shown to protect these flocks from field strains (Kleven et al., 1990). The F-strain *M. gallisepticum* vaccine, however, is pathogenic to turkeys and is not approved or licensed for use in poultry other than commercial layers. In a controlled study in biological isolation units, early vaccination of commercial layers with F-strain *M. gallisepticum* did not adversely affect EP (Branton et al., 1997). Anecdotal evidence from producers that have used F-strain *M. gallisepticum* has also shown that no adverse effects on EP occurred when F-strain *M. gallisepticum* vaccinations were provided prelay (personal communication, Jack Self, vice president of operations, Cal Maine Foods Inc., Jackson, MS). However, some field studies have determined that F-strain *M. gallisepticum* vaccination can reduce EP when compared with *Mycoplasma*-free birds (Carpenter et al., 1981; Mohammed et al., 1987; Branton et al., 1988). More recently, apathogenic ts11-strain *M. gallisepticum* and 6/85-strain *M. gallisepticum* live vaccines have been licensed for use in layer chickens. These vaccines show virtually no bird-to-bird transmission, but have not been proven to displace wild-type *M. gallisepticum* (Kleven, 1998). Furthermore, these strains may not confer continued protection, as does the F-strain throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987). More testing is needed to determine whether combinations of vaccines can lessen the adverse impact of prelay F-strain *M. gallisepticum* vaccinations on layer performance when given alone. Therefore, the objective of the current study was to determine the effects of prelay ts11-strain *M. gallisepticum* inoculations and time-specific F-strain *M. gallisepticum* inoculation overlays administered during lay on the performance characteristics of commercial laying hens.

MATERIALS AND METHODS

Pullet Housing and Management

Two trials were performed with 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 *M. gallisepticum* and *Mycoplasma synoviae* (USDA-Animal and Plant Health Inspection Service-Veterinary Services, 2003). Chickens were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 5 wk of age, 20 randomly selected chickens were tested for antibodies to both *M. gallisepticum* and *M. synoviae* by using both the serum plate agglutination (**SPA**) and the hemagglutination-inhibition tests (Yoder, 1975), and swabs were obtained from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 thallium acetate and 10^6 IU of penicillin-G/mL. Tubes were incubated at 37°C for 30 d or until the phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based agar and incubated at 37°C. Colonies with morphology suggestive of Mycoplasma species were examined by an agar plate fluorescent antibody (**FA**) test (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-F-strain *M. gallisepticum* polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Pullets were placed on clean dry litter in a conventional house until 10 wk of age. A daily artificial lighting schedule followed a 13 L:11 D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lx. Feed and water were provided for ad libitum consumption in each trial. At 10 wk of age, 11 pullets were randomly selected and placed in each of 16 negative-pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Hen numbers were reduced to 10 per unit at point of lay (22 wk of age) so that bird density was 0.116 m^2/bird for the duration of each trial. At 18 wk of age, the length of the artificial lighting schedule was increased by 15 min/d until a cycle of 16 h and 15 min of light per 7 h and 45 min of dark was achieved. Chickens were maintained on that schedule through the remainder of each of the trials. For the entirety of each trial, chickens had ad libitum access to feed and water. Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages and calculated analyses of the diets were as described by Burnham et al. (2002). No medications were administered during either trial.

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 (controls) received no *M. gallisepticum* inoculation but were sham-inoculated via eye drop in the right eye with sterile Frey's media. Treatment 2 contained birds that were eye-drop vaccinated in the right eye with ts11-strain *M. gallisepticum* at 10 wk of age (**ts11/10**). Birds belonging to treatment 3 received ts11-strain *M. gallisepticum* via eye drop at 10 wk of age followed by a 22-wk overlay vaccination via eye drop in the left eye with F-strain *M. gallisepticum* (**ts11/10-F/22**). Treatment 4 consisted of birds given ts11 strain *M. gallisepticum* at 10 wk of age via eye drop in the right eye followed by a 45-wk overlay vaccination of F-strain *M. gallisepticum* via eye drop in the left eye (**ts11/10-F/45**).

Data Collection

All data collected before wk 22 were designated as belonging to age interval I; all data collected from wk 22 to 44 were designated as belonging to age interval II; and all data collected from wk 45 to 57 were designated as belonging to interval III. Individual BW was recorded at 20 wk (interval I), at 24, 32, and 43 wk (interval II), and at 47 and 56 wk of age (interval III) in both trials 1 and 2.

Egg production was recorded daily and analyzed weekly (weekly EP) from wk 23 (when control treatment EP reached approximately 10%) through wk 44 (interval II) and from wk 45 through 55 (interval III), and was expressed as percent hen-day EP. Furthermore, the total number of eggs produced per hen (total hen EP) from onset of lay through 55 wk (across all 3 intervals), and within interval II (wk 22 to 44) and interval III (wk 45 to 55) separately, were likewise determined. To include all eggs that were laid before 10% EP, the calculation of total hen EP across all 3 intervals was initiated when the first egg was laid (onset of lay). Age of onset of lay ranged between 18 and 21 wk. Total hen EP was calculated as the total daily numbers of eggs produced as a percentage of the total daily numbers of hens for each replicate group.

Beginning on wk 23 (when control treatment EP reached approximately 10%), eggs were collected 2 d/wk to determine egg weight (**EW**), eggshell breaking strength (**ESS**), and percentage incidences of eggshell pimpling (**ESP**), egg blood spots (**EBS**), and egg meat spots (**EMS**). Egg weight, ESS, ESP, EBS, and EMS determinations were recorded weekly in both trials from 23 to 57 wk of age. These determinations were made on all the same eggs that were produced within the 2-d time period. The ESS of each egg was determined by the technique described by Reece and Lott (1976), and ESP, EBS, and EMS incidences were determined by using the methods described by Branton et al. (1997). For determination of eggshell weight per unit of eggshell surface area (**SWUSA**), a total of 10 eggs per pen were weighed at 20, 24, 32, 43, and 46 wk of age in both trials. The eggs used for SWUSA measurements were different from the eggs used for determination of EW, ESS, ESP, EBS, and EMS. Determination of SWUSA was according to the procedure described by Peebles et al. (1994). All the above egg and eggshell determinations were recorded on the same day that the eggs were collected.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was used. Data before wk 22 (age interval I), from wk 22 to 44 (age interval II), and from wk 45 to 57 (age interval III) 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 each age interval were subjected to a repeated measures analysis if parameters were examined at multiple age periods in an age interval. Otherwise, data (i.e., total hen EP) obtained within and across age intervals was subjected to 1-way ANOVA.

In the first age interval, the control group and the ts11/10 treatment group were compared. In the second age interval, control birds and those having had ts11/10 and ts11/10-F/22 inoculations were compared. In the third age interval, the control, ts11/10, ts11/10-F/22, and ts11/10-F/45 groups were compared. 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 by using the MIXED procedure of SAS (SAS Institute, 2003).

RESULTS AND DISCUSSION

All initial mycoplasmal cultures obtained from 5-wk-old pullets, as well as SPA tests, were negative for both *M. gallisepticum* and *M. synoviae.* As described by Zander (1984), *M. gallisepticum* is already established on many multiage farms, and transmission from mature hens to replacement pullets ensures its existence. Therefore, in this study, birds were also later tested to ensure the absence of disease transmission between treatment groups. The aforementioned cultures and SPA tests were repeated at 27 and 51 wk. Throughout the 2 trials, these sample tests on all 4 treatment groups showed that control birds remained *Mycoplasma* free (negative for both *M. gallisepticum* and *M. synoviae*), whereas the 3 vaccinated groups resulted in positive broth cultures, with FA and SPA results being positive for *M. gallisepticum* and negative for *M. synoviae.* The SPA and FA tests confirmed systemic infections in *M. gallisepticum*-inoculated birds, whether inoculated with the ts11- or F-strain of *M. gallisepticum.* Nevertheless, there was no significant difference in mortality between *M. gallisepticum*-free and *M. gallisepticum*-inoculated birds in either trial. More specifically, no significant age or treatment main effects or age by treatment interactions were found for bird mortality in any of the 3 age intervals examined. The few mortalities recorded had no apparent relationship to treatment application.

In large commercial multiage layer facilities, where depopulation followed by disinfection is impractical, vaccination programs are the best protection (Bermudez and Kalbac, 1988). Vaccination for diseases in animals is commonly practiced where the disease is endemic (Barile, 1985). Furthermore, early vaccination allows for the early development of immunity to *Mycoplasmas* before EP begins. Therefore, in 1 of the treatment groups of this study, an early prelay vaccination was used, and in 2 of the other treatments, the early vaccination was used in conjunction with later overlay vaccinations to confer continued protection during lay. Despite these treatment applications, only a main effect ($P \leq 0.04$) attributable to hen age for BW was observed in interval II; hen BW at wk 24, 32, and 43 were 1.34, 1.40, and 1.45 kg, respectively (pooled SEM = 0.022). Hen BW at wk 23 and 43 were significantly greater than that at wk 24, but BW at wk 32 and 43 were not significantly different. There was no treatment effect at wk 20 in interval I, and there was no significant treatment main effect or age by treatment interaction in interval II. In addition, there were no significant age or treatment main effects or an age by treatment interaction for BW in interval III. These BW data are consistent with those reported by Burnham et al. (2002), Peebles et al. (2007), and Viscione et al. (2008). Burnham et al. (2002) found no effect of a 12-wk F-strain *M. gallisepticum* inoculation on subsequent BW, Peebles et

Table 1. Total hen egg production of commercial layers in treatment groups of sham-inoculated control (control), ts11 *Mycoplasma gallisepticum* at 10 wk (ts11/10), ts11 *M. gallisepticum* at 10 wk with a 22-wk F-strain *M. gallisepticum* overlay (ts11/10-F/22), and ts11 *M. gallisepticum* at 10 wk with a 45-wk F-strain *M. gallisepticum* overlay (ts11/10-F/45) within age intervals II (wk 22 to 44) and III (wk 45 to 55)[1]

Inoculation treatment	Age interval	
	II[2]	III[3]
	(%)	
Control	85.2	73.4
ts11/10	81.9	74.5
ts11/10-F/22	81.3	63.1
ts11/10-F/45	—	70.2

[1]n = 4 replicate units for calculation of mean within each interval and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 2.63.

[3]SEM based on pooled estimate of variance = 3.64.

Table 2. Egg weight of commercial layers in treatment groups of sham-inoculated control (control), ts11 *Mycoplasma gallisepticum* at 10 wk (ts11/10), and ts11 *M. gallisepticum* at 10 wk with a 22-wk F-strain *M. gallisepticum* overlay (ts11/10-F/22) at 27, 37, and 38 wk of age[1,2]

Inoculation treatment	Age (wk)		
	27	37	38
	(g)		
Control	50.7[b]	56.3[a]	56.8[a]
ts11/10	50.8[b]	53.9[b]	56.0[a]
ts11/10-F/22	53.1[a]	55.9[a]	52.9[b]

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

[1]n = 4 replicate units for calculation of the mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.75.

al. (2007) showed that BW was not affected by inoculation of F-strain *M. gallisepticum* at either 12 or 22 wk of age, and Viscione et al. (2008) noted no significant effects of F-strain *M. gallisepticum* inoculation at either 22 or 45 wk of age on layer hen BW. No previous research has reported the effects of prelay ts11-strain *M. gallisepticum* vaccinations on layer BW.

A hen age main effect was observed for weekly EP in interval III (wk 45 to 55; $P \leq 0.01$; data not shown); however, no significant treatment main effect or age by treatment interaction was observed for weekly EP in interval III. In addition, no significant effects of any kind were observed for weekly EP in interval II. Furthermore, total hen EP across lay (intervals I to III) and total hen EP within interval II and within interval III were not significantly affected by treatment. Although total hen EP within intervals II and III and across all 3 intervals was not affected by treatment, treatment means within interval II and interval III are provided in Table 1 for observation. In addition, means for total hen EP across lay for the control, ts11/10, ts11/10-F/22, and ts11/10-F/45 treatments were 76.8, 76.8, 70.8, and 72.2%, respectively (pooled SEM = 2.36). In trials conducted by Branton et al. (1997), no negative effects were noted in EP or EW for birds that were challenged with F-strain *M. gallisepticum* before lay, when compared with control birds. The results of Branton et al. (1997) support other research previously conducted by Yoder (1978, 1991) and Mohammed et al. (1987), who reported that F-strain *M. gallisepticum* vaccination can reduce the negative impact of natural *M. gallisepticum* infections in layer flocks. However, in studies reported by Burnham et al. (2002), in which egg-laying hens were inoculated at 12 wk of age with F-strain *M. gallisepticum*, it was reported that there was a decrease in total EP and that birds laid their first eggs approximately 1 wk later than controls that received only sham inoculations. Furthermore, Peebles et al. (2008) noted that F-strain *M. gallisepticum* inoculations given at either 12 or 22 wk of age decreased EP at the beginning of lay.

The lack of a prelay ts11-strain *M. gallisepticum* inoculation influence on EP in the current study suggests that the possible negative effect of a prelay F-strain *M. gallisepticum* inoculation on EP, as observed by Burnham et al. (2002) and Peebles et al. (2008), might be avoided by using a ts11/10 prelay vaccination program. This suggestion is supported by results from a study similar to the current one conducted by Branton et al. (2000), in which a prelay inoculation of ts11-strain *M. gallisepticum* alone was used. The authors determined in that study that there were no significant differences in EP or other egg and eggshell quality parameters between the ts11-strain *M. gallisepticum*-vaccinated and control birds, which indicated that a prelay ts11 vaccine had no negative impact on overall layer performance.

In a study reported by Branton et al. (1988), in which layer hens were inoculated with F-strain *M. gallisepticum* at 45 wk of age, there was a significant decrease in EP compared with control birds that were not vaccinated. This was in agreement with earlier work published by Carpenter et al. (1981), who found that layers maintained free from *M. gallisepticum* infection laid an average of 8.7 eggs/hen housed more than did flocks that were vaccinated with F-strain *M. gallisepticum* at 45 wk of age. The results of Branton et al. (1988), Carpenter et al. (1981), and Peebles et al. (2008) demonstrate further potential negative impacts of 22- and 45-wk F-strain *M. gallisepticum* inoculations on performance. Because EP was not affected by the use of ts11/10-F/22 or ts11/10-F/45 vaccine treatments in the current 2 trials, it is also suggested that a prelay ts11/10 vaccination may help to prevent a significant drop in hen day EP when F-strain *M. gallisepticum* vaccination overlays are given at either 22 or 45 wk. A prelay ts11/10 inoculation might serve to reduce the negative impacts of F-strain *M. gallisepticum* inoculations given during lay.

An age by treatment interaction was observed for EW ($P \leq 0.04$) in interval II (Table 2). At wk 27, EW was significantly larger in the ts11/10-F/22 treatment group compared with the control and ts11/10 treatment groups. This was opposite that at wk 38, where the control and ts11/10 treatment birds had greater EW than the ts11/10-F/22 birds. At wk 37, the control and ts11/10-F/22 birds had a higher EW than the ts11/10 treatment group. There were no significant age or treatment main effects or age by treatment interactions in interval III. It has been noted that EW was unaffected by inoculations with ts11-strain *M.*

Table 3. Eggshell pimpling incidence of commercial layers in treatment groups of sham-inoculated control (control), ts11 *Mycoplasma gallisepticum* at 10 wk (ts11/10), ts11 *M. gallisepticum* at 10 wk with a 22-wk F-strain *M. gallisepticum* overlay (ts11/10-F/22), and ts11 *M. gallisepticum* at 10 wk with a 45-wk F-strain *M. gallisepticum* overlay (ts11/10-F/45) at 53, 56, and 57 wk of age[1,2]

	Age (wk)		
Inoculation treatment	53	56	57
	(%)		
Control	0.290[b]	0.223[b]	0.365[a]
ts11/10	0.389[ab]	0.273[b]	0.280[a]
ts11/10-F/22	0.301[b]	0.250[b]	0.268[ab]
ts11/10-F/45	0.502[a]	0.410[a]	0.143[b]

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

[1]n = 4 replicate units for calculation of the mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.0600.

Table 4. Egg blood spot incidence of commercial layers in treatment groups of sham-inoculated control (control), ts11 *Mycoplasma gallisepticum* at 10 wk (ts11/10), ts11 *M. gallisepticum* at 10 wk with a 22-wk F-strain *M. gallisepticum* overlay (ts11/10-F/22), and ts11 *M. gallisepticum* at 10 wk with a 45-wk F-strain *M. gallisepticum* overlay (ts11/10-F/45) at 54 and 56 wk of age[1,2]

	Age (wk)	
Inoculation treatment	54	56
	(%)	
Control	0.000[b]	0.041[b]
ts11/10	0.016[b]	0.018[b]
ts11/10-F/22	0.183[a]	0.025[b]
ts11/10-F/45	0.018[b]	0.161[a]

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

[1]n = 4 replicate units for calculation of mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.0300.

gallisepticum at 10 wk (Branton et al., 2000), F-strain *M. gallisepticum* at 12 wk (Burnham et al., 2002), F-strain *M. gallisepticum* at 12 and 22 wk (Peebles et al., 2008), and F-strain *M. gallisepticum* at 22 and 45 wk (Viscione et al., 2008). Branton et al. (1988) did find that F-strain *M. gallisepticum* inoculated at 45 wk increased EW over a 15-wk period, but this was noted in only 1 of 2 trials. In addition, Burnham et al. (2002) found that the percentage of eggs belonging to the USDA undersized egg category decreased at 19 wk but later increased at 20 and 21 wk after a 12-wk F-strain *M. gallisepticum* inoculation. The inconsistent results of these aforementioned studies do not convincingly demonstrate that EW is susceptible to the various inoculation regimens described involving the ts11- and F-strains of *M. gallisepticum.* However, despite the apparent innocuousness of a ts11/10 vaccination on EP, consideration should be given to possible subsequent effects that a ts11/10 vaccination might have on EW. This is suggested in this study because of the reduction in EW of the ts11/10 treatment group relative to the control group at wk 37. Furthermore, the current data indicate that an overlay of F-strain *M. gallisepticum* at 22 wk may lead to respective increases and decreases in EW at 27 and 38 wk in birds that had been given ts11-strain *M. gallisepticum* vaccinations at 10 wk.

The effects of prelay and wk 22 F-strain *M. gallisepticum* inoculations on ESS, ESP, EBS, and EMS have not been investigated previously. Nevertheless, F-strain *M. gallisepticum* vaccinations at 45 wk (Branton et al., 1988) and ts11-strain *M. gallisepticum* vaccinations at 10 wk of age (Branton et al., 2000) have been reported to have no effect on these same parameters. However, in the current study, significant age by treatment interactions were observed for ESP ($P \leq 0.05$; Table 3) and EBS ($P \leq 0.05$; Table 4) in interval III. At wk 53, ESP was significantly higher for the ts11/10-F/45 treatment group than for the control or ts11/10-F/22 treatment groups. The ESP of the ts11/10 treatment group was not significantly different from the other 3 treatment groups. At wk 56, the ts11/10-F/45 treatment group had a significantly higher ESP than the control, ts11/10, or ts11/10-F/22 treatment group. Conversely, at wk 57, the ts11/10-F/45 treatment group was significantly lower than the control or ts11/10 treatment group; however, the ts11/10-F/22 group was not significantly different from the other 3 groups. For EBS at wk 54, the incidence was higher for the ts11/10-F/22 treatment group when compared with the control, ts11/10, and ts11/10-F/45 treatment groups. However, at wk 56, the ts11/10-F/45 treatment group had a significantly higher incidence of EBS than did the other 3 groups. There were no significant effects of any kind for ESP or EBS in interval II, for ESS or EMS in interval II or III, or for SWUSA in any of the 3 time intervals. The egg and eggshell quality parameters selected for these trials reflected the functionality of the ovary and specific segments of the oviduct as described by Branton et al. (2000). However, ESS, EMS, and SWUSA were not affected, and EW, ESP, and EBS were affected inconsistently by the combined vaccine treatments.

In conclusion, although possible effects of the combined vaccinations on EW, ESP, and EBS should be considered, they had no recurrent negative impact on egg and eggshell quality in the present study. Furthermore, the prelay ts11/10 vaccination appeared to prevent a decrease in EP when F-strain *M. gallisepticum* was given during lay. These results suggest that an overlay of F-strain *M. gallisepticum* on prelay ts11-strain *M. gallisepticum*-vaccinated birds does not adversely affect layer performance. Each of the vaccine treatments examined in this study had specific strengths and weaknesses. As noted in earlier work, the individual use of ts11- or F-strain *M. gallisepticum* vaccinations may respectively have an adverse effect on EP or may not confer continued protection against field strain *M. gallisepticum* infections. However, the ts11- and F-strain *M. gallisepticum* vaccine treatment combinations may overcome some of the weaknesses that prelay vaccines of ts11- or F-strains of *M. gallisepticum* may have if given alone. The prelay ts11-strain and lay F-strain *M. gallisepticum* vaccine combination shows promise as research continues to develop new and better vaccine protocols to eliminate the negative impacts of *Mycoplasma* vaccination. It is suggested that to better establish the protective benefits of a dual vaccination ap-

proach, this study should be followed by one using a virulent *M. gallisepticum* challenge during lay.

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