Transmissibility of Live Mycoplasma gallisepticum Vaccine Strains ts-11 and 6/85 from Vaccinated Layer Pullets to Sentinel Poultry

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SUMMARY. In separate trials, layer pullets were vaccinated with Mycoplasma gallisepticum (MG) strain 6/85 or strain ts-11 commercially produced live vaccines. For a 15-wk postvaccination (PV) period, vaccinates were commingled with unvaccinated pullets and were in indirect contact with sentinel groups of pullets, broiler breeders, turkey breeders, or meat turkeys in adjoining pens. Infectivity and transmissibility of vaccine strains were determined by tracheal culture and serology at 1 wk followed by 3-wk intervals PV. Strain 6/85 was recovered from 0%–20% of vaccinates, but not from commingled pullets or sentinel birds. Strain ts-11 was recovered from 60%–90% of vaccinates and 0%–40% of commingled pullets but not from any of the sentinel birds. No birds in the 6/85 vaccine trial tested positive for MG antibodies by serology. MG enzyme-linked immunosorbent assays detected positive responses in ts-11 vaccinates (range = 10%–70%) at 42, 63, 84, and 105 days PV, and commingled pullets (10%) at 84 and 105 days PV. MG serum plate agglutination tests detected positive responses in 90% and 20% of ts-11 vaccinates at 42 and 105 days PV, respectively, and commingled pullets (10%) at day 42 PV. Clinical signs, morbidity, or mortality suggestive of pathogenic MG infection were not observed in any bird during either trial, and no gross lesions were observed at necropsy. Random amplified polymorphic DNA analysis was capable of distinguishing each of the vaccinal strains 6/85 and ts-11 from each other by their distinct DNA banding patterns.

RESUMEN. Transmisibilidad de las cepas vacunales vivas de Mycoplasma gallisepticum ts-11 y 6/85, de pollonas de reemplazo a aves centinelas.

En experimentos separados, se vacunaron pollonas de reemplazo con vacunas comerciales vivas de Mycoplasma gallisepticum (MG) con las cepas vacunales 6/85 o ts-11. Después de un período de 15 semanas postvacunación, las pollonas vacunadas fueron mezcladas con las no vacunadas y estuvieron en contacto indirecto con grupos de aves centinelas que se alojaron en locales adjuntos e incluyeron pollonas de reemplazo, reproductoras pesadas, paves reproductores o paves comerciales de engorde. La capacidad de infección y transmisión de las cepas vacunales se determinó mediante cultivos traqueales y serología a la primera semana postvacunación y luego con intervalos de cada 3 semanas. La cepa 6/85 se reasiló del 0%–20% de las aves vacunadas, pero no a partir de las aves que se juntaron o de las aves centinelas. La cepa ts-11 se reasiló del 60%–90% de las aves vacunadas y del 0%–40% de las que se juntaron pero no se aisló de ninguna de las aves centinelas. Ninguna de las aves vacunadas con la cepa 6/85 resultó serológicamente positiva contra MG. Mediante la prueba de inmunensayo con enzimas asociadas (ELISA) se detectaron respuestas positivas en las aves vacunadas con la cepa ts-11 variando del 10%–70% a los 42, 63, 84 y 105 días postvacunación, y en el 10% de las aves que se juntaron a los 84 y 105 días postvacunación. La
prueba de sueroaglutinación en placas contra MG detectó respuestas positivas en el 90% y 20% de las aves vacunadas con la cepa ts-11 a los 42 y 105 días postvacunación, respectivamente, y en el 10% a los 42 días postvacunación en las aves que se juntaron con las vacunadas. No se observaron signos clínicos, morbilidad o mortalidad que sugirieran una infección patógena de MG en ninguna de las aves durante los experimentos, y no se observaron lesiones macroscópicas a la necropsia. El análisis del ácido desoxirribonucleico (ADN) polimórfico pudo diferenciar cada una de las cepas vacunales, la 6/85 y la ts-11, de acuerdo con sus patrones característicos de las bandas de ADN.

Key words: Mycoplasma gallisepticum, vaccine, chicken, turkey, random amplified polymorphic DNA.

Abbreviations: ELISA = enzyme-linked immunosorbent assay; FMS = Frey's broth medium with 15% swine serum; HI = hemagglutination inhibition; KPL = Kirkegaard & Perry Laboratories, Inc.; MG = Mycoplasma gallisepticum; MM = Mycoplasma meleagris; MS = Mycoplasma synoviae; NVSL = National Veterinary Services Laboratory; PCR = polymerase chain reaction; PV = postvaccination; RAPD = random amplified polymorphic DNA; SPA = serum plate agglutination

\textit{Mycoplasma gallisepticum} (MG) is an important pathogen of poultry worldwide and is responsible for substantial economic losses from decreased egg production and hatchability, downgrading and condemnations of carcasses, and decreased feed efficiency [20]. Strategies to minimize or eliminate the impact of MG infection in commercial poultry include surveillance, control, and eradication programs; antimicrobials; and vaccination [20]. Live F strain MG vaccine has been used extensively in layer pullets to reduce losses that otherwise occur when they are moved to multiple-age laying complexes containing older birds infected with MG [20]. F strain-vaccinated laying hens have better production compared to unvaccinated hens in flocks with enzootic MG, but do not produce as well as MG-clean flocks [2,15]. Furthermore, the vaccine F strain of MG is pathogenic for turkeys following experimental infection [14], and has been associated with MG outbreaks in meat and breeder turkeys under field conditions [13]. Recently, MG strains 6/85 and ts-11 have been produced commercially as live whole-cell vaccines. Both vaccines are poorly transmitted from vaccinated to unvaccinated birds and possess little or no virulence for chickens and turkeys [1,4,19], suggesting they should be safe for vaccinated flocks and pose little or no threat of infection and disease for susceptible flocks of any poultry type.

Serology is an extremely useful tool for monitoring and diagnosis of MG in flock control programs. For unvaccinated flocks, MG-positi-
MATERIALS AND METHODS

Chickens and turkeys. Commercial chickens and turkeys were individually identified with wing bands and placed in pens on concrete floors covered with pine shavings in isolation (biosafety level 2) rooms. Chickens and turkeys were given access to unmedicated feed appropriate for the type and age of bird, and water ad libitum. The birds were on a 12-hr light–dark cycle for weeks 1–7, and a 17-hr light–7-hr dark cycle for weeks 8–15 to stimulate egg production in the breeder and layer birds. In each of two trials, there were three groups of 10-wk-old layer pullets, one group of 14-wk-old broiler breeders, one group of 22-wk-old turkey breeder hens, and one group of 4-wk-old straight-run meat turkeys. Each group consisted of 10 birds, and all were tested in the prevaccination period for Mycoplasma spp. by tracheal culture and enzyme-linked immunosorbent assay (ELISA) serology.

Isolation and identification of MG. Tracheal swabs for mycoplasma culture were inoculated into Frey’s broth medium (6) supplemented with 15% swine serum (FMS) and incubated at 37 C for up to 3 wk. At 14 days postsampling, all broths were passed into fresh FMS broth and onto FMS agar media and incubated at 37 C for 7 days. Broths showing fermentation were passed into fresh FMS broth and onto FMS agar media and incubated at 37 C for 7 days. Mycoplasma colonies on FMS agar were identified as MG by direct immunofluorescence (IF) (12) using fluorescein-conjugated rabbit antiserum provided by S. Kleven (Department of Avian Medicine, University of Georgia, Athens, Ga.).

ELISA. All sera were tested for antibodies to MG using a commercial test kit (Kirkegaard & Perry Laboratories Inc., KPL, Gaithersburg, Md.) according to the manufacturer’s directions. Sera collected from prevaccination and terminal samplings were tested for Mycoplasma synoviae (MS), and for Mycoplasma meleagridis (MM) (turkeys only) by ELISA (KPL). Sera were considered positive by ELISA if the sample-to-positive ratio was ≥0.6.

Serum plate agglutination (SPA). Sera from postvaccination (PV) samplings were tested for MG antibodies using the SPA test with commercially available antigen (Intervet, Inc., Millsboro, Del.) according to the manufacturer’s directions. MG-positive chicken and turkey sera obtained from the National Veterinary Services Laboratory (NVSL, Ames, Iowa) and in-house negative sera were used as controls.

Hemagglutination inhibition (HI). HI assays to detect MG antibodies were performed on PV serum samples using four hemagglutination units (12) of antigen obtained from NVSL. Positive MG serum obtained from NVSL and in-house MG-negative serum were used as controls.

- Pullets
- Vac. Pullets & Unvac. Pullets
- Turkey Breeders
- Meat Turkeys

Fig. 1. Pen (chain link, 4 ft high) configuration for each of two trials within biosafety level 2 isolation rooms. Central pen containing vaccinated and unvaccinated (commingled) pullets was covered with wire. Adjoining pens contained sentinel pullets, broiler breeders, turkey breeders, and meat turkeys. These birds could contact vaccines through the fence but had separate feeders and waterers.

rum were used as controls. Samples were considered positive if HI titers were 1:80 or greater.

MG vaccination. Prior to vaccination, each group of designated vaccines was moved to a separate isolation (biosafety level 2) room and placed in stainless steel cages. MG strain 6/85 (Mycovac L, Intervet, Inc.) and strain ts-11 (Select Laboratories, Inc., Gainesville, Ga.) live vaccines were used to vaccinate two groups of 10 layer pullets by representatives of each manufacturer according to the manufacturer’s instructions. Strain 6/85 was administered by using a paint sprayer and compressor (40 psi) exposing the birds to a fine spray (particle size less than 40 μm). Strain ts-11 was administered by placing one drop on the eye and allowing the drop to spread over the eye before releasing the bird. Each group of vaccines remained in their respective isolation rooms overnight, and was returned the following day to the central pen in each of two isolation (biosafety level 2) rooms (Fig. 1).

Experimental design. In a separate isolation room for each trial, conjoining pens of equal area were constructed of chain link fence (Fig. 1). The central pen housed both vaccinated and unvaccinated (commingled) pullets. Pens adjoining the central pen contained sentinel groups (pullets, broiler breeders, turkey breeders, and meat turkeys). Vaccines and commingled pullets shared the same feeders and waterers within the central pen, whereas sentinel birds had separate feeders and waterers in each pen. Wing feathers on all birds were trimmed periodically during the trials to inhibit flight. In addition, the central pen containing vaccines and commingled pullets was covered with wire mesh to eliminate any possibility of bird movement into or out of the pen.

Birds were observed daily for clinical signs, morbidity, and mortality throughout the trial. Blood and tracheal swabs were taken from all birds for MG serology (ELISA, HI, and SPA) and culture, respectively, at 3-wk intervals PV. In addition, layer pullets
were examined for the presence of MG by serology and tracheal culture at 7 days PV.
At 15 wk PV, all birds were sampled for mycoplasma serology and culture. Birds were euthanized with Beuthanasia-D (Schering Plough, Kenilworth, N. J.), necropsied, and examined for gross lesions.

**RAPD.** MG isolates from PV samplings of vaccinated and commingled pullets were tested for strain identification using a RAPD test as previously described (5), with some modifications. Briefly, 3 μl of DNA extracted from MG isolates was added to reaction mixes containing three custom-made primers (Gibco BRL, Life Technologies, Grand Island, N.Y.). Sequences of the three primers designated S1OLA03, M13F, and M16SPCR5 were 5'-CATACGACATAGGGCAA-3', 5'-GTAAAACAGCGGC-3', and 5'-AGGCCAGCATAGGGAAT-3', respectively (5). PCR were amplified in a thermal cycler (Perkin Elmer, Norwalk, Conn.). Amplified DNA was separated using 2% agarose gel (Agarose NA Pharmacia, Uppsala, Sweden) electrophoresis, visualized with ultraviolet (UV) light, and photographed (Polaroid Corp., Cambridge, Mass.). Photographs were digitized, and the figures shown were composed using Photoshop (Adobe Systems Inc., Mountain View, Calif.) and QuarkXpress (Quark Inc., Denver, Colo.). RAPD banding patterns of MG isolates were compared with each other and with samples of each vaccine strain used in these trials.

### RESULTS

**Clinical signs, morbidity, and mortality.** There were no clinical signs, morbidity, or mortality suggestive of pathogenic MG infection observed in any bird during either trial, and no gross lesions were observed at necropsy.

**MG culture.** MG was not recovered from any bird at prevaccination sampling. MG culture results for the 6/85 trial are shown in Table 1. MG was recovered from two vaccinated pullets at each sampling from days 21 to 105 PV. The same two pullets were culture positive for MG at above samplings except at day 63 PV, when a sample from one of the two pullets had contaminant overgrowth. MG was not recovered from any commingled pullet or sentinel bird during this trial.

MG culture results for the ts-11 trial are shown in Table 2. MG was recovered at moderate to high rates from vaccinated pullets at

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**Table 1. *Mycoplasma gallisepticum* (MG) tracheal culture results of strain 6/85 vaccination trial.**

<table>
<thead>
<tr>
<th>Group (n = 10)</th>
<th>% Positive birds on day postvaccination*</th>
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<tbody>
<tr>
<td></td>
<td>-6</td>
</tr>
<tr>
<td>Vaccinated pullets*</td>
<td>0</td>
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<tr>
<td>Commingled pullets</td>
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<tr>
<td>Sentinel pullets</td>
<td>0</td>
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<tr>
<td>Sentinel broiler breeders</td>
<td>0</td>
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<tr>
<td>Sentinel turkey breeders</td>
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<tr>
<td>Sentinel meat turkeys</td>
<td>0</td>
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</tbody>
</table>

*MG identified by direct immunofluorescence.

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**Table 2. *Mycoplasma gallisepticum* (MG) tracheal culture results of strain ts-11 vaccination trial.**

<table>
<thead>
<tr>
<th>Group (n = 10)</th>
<th>% Positive birds on day postvaccination*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-8</td>
</tr>
<tr>
<td>Vaccinated pullets*</td>
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<td>Commingled pullets</td>
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<tr>
<td>Sentinel pullets</td>
<td>0</td>
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<tr>
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<td>Sentinel turkey breeders</td>
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<td>Sentinel meat turkeys</td>
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*MG identified by direct immunofluorescence.

*Vaccinated by eyedrop.

*Not tested.

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Table 3. *Mycoplasma gallisepticum* (MG) enzyme-linked immunosorbent assay (ELISA) results of strain ts-11 vaccination trial.

<table>
<thead>
<tr>
<th>Group (n = 10)</th>
<th>% Positive birds on days postvaccination&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−8</td>
</tr>
<tr>
<td>Vaccinated pullets&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
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<tr>
<td>Commingled pullets</td>
<td>0</td>
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<td>Sentinel pullets</td>
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<tr>
<td>Sentinel turkey breeders</td>
<td>0</td>
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<tr>
<td>Sentinel meat turkeys</td>
<td>0</td>
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</tbody>
</table>

<sup>a</sup>Kirkegaard & Perry Laboratories, Inc., ELISA sample-to-positive ratio ≥0.6.
<sup>b</sup>Vaccinated by eyedrop.
<sup>c</sup>Not tested.

Each sampling date PV (days 7–105 PV) and was recovered at low to moderate rates from commingled pullets at each sampling from days 21 to 105 PV. One commingled pullet sample had contaminant overgrowth at days 84 and 105 PV. MG was not recovered from any sentinel bird at any sampling during this trial.

**Serology.** Serum antibodies to MG were not detected by ELISA from any bird at prevaccination sampling. None of the birds in the 6/85 trial tested positive for MG antibodies by ELISA, SPA, and HI tests at any sampling date PV.

ts-11-Vaccinated pullets showed a progressive increase in MG seropositive rates by ELISA from days 42 to 105 PV (Table 3). Each ts-11-vaccinated pullet was MG seropositive by ELISA at one or more of the above samplings. The same commingled pullet was positive for MG by ELISA at the final two sampling dates. The MG SPA test detected 90% and 20% seroconversion in ts-11-vaccinated pullets on days 42 and 105 PV, respectively (Table 4). One commingled pullet was MG positive by SPA test on day 42 PV. No bird in the ts-11 sentinel groups was positive for MG antibodies by ELISA and SPA test at any sampling during the trial. The HI test yielded no positive results for MG antibodies for any bird in the ts-11 trial. No birds were seropositive for MS or MM (turkeys only) at prevaccination and terminal samplings.

**RAPD.** RAPD profiles of MG vaccine strains 6/85 and ts-11 (Figs. 2, 3) showed notable differences in their respective banding patterns. RAPD analysis of MG strain 6/85 vaccine and MG isolates from strain 6/85-vaccinated pullets at various days PV are shown in Fig. 2. MG isolates showed nearly identical banding patterns to MG vaccine strain 6/85 and to each other. RAPD profiles of MG strain ts-11 vaccine and MG isolates from strain ts-11-vaccinated and commingled pullets at various days PV are shown in Fig. 3. MG iso-

Table 4. *Mycoplasma gallisepticum* (MG) serum plate agglutination (SPA) results of ts-11 vaccination trial.

<table>
<thead>
<tr>
<th>Group (n = 10)</th>
<th>% Positive birds on day postvaccination&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>−8</td>
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<tr>
<td>Vaccinated pullets&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Commingled pullets</td>
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<tr>
<td>Sentinel turkey breeders</td>
<td>NT</td>
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<tr>
<td>Sentinel meat turkeys</td>
<td>NT</td>
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</table>

<sup>a</sup>SPA test = National Veterinary Services Laboratory antigen, four hemagglutination units.
<sup>b</sup>Vaccinated by eyedrop.
<sup>c</sup>Not tested.
Fig. 2. Random amplified polymorphic DNA profiles of *Mycoplasma gallisepticum* (MG) strain 6/85 vaccine and MG isolates from strain 6/85-vaccinated pullets at various days postvaccination (PV). Lane 1, DNA size standards (Bio-Rad Laboratories, Hercules, Calif.); lane 2, strain 6/85 vaccine; lane 3, negative control (reaction mixes without DNA); lane 4, isolate from vaccinated pullet 14R at day 21 PV; lane 5, isolate from vaccinated pullet 14R at day 42 PV; lane 6, isolate from vaccinated pullet 14R at day 84 PV; lane 7, isolate from vaccinated pullet 18R at day 21 PV; lane 8, isolate from vaccinated pullet 18R at day 42 PV; lane 9, isolate from vaccinated pullet 18R at day 63 PV. DNA sizes in kilobases are shown on the left.

Sera had nearly identical banding patterns to MG vaccine ts-11 and to each other.

**DISCUSSION**

In the present study, MG vaccine strains 6/85 and ts-11 did not cause any clinical signs or pathologic lesions suggestive of MG infection and appeared to be safe in vaccines. These findings are in agreement with others who demonstrated that these two vaccines produced little or no vaccination reaction in chickens and/or turkeys (1,4,19).

MG was isolated infrequently from tracheal swabs of 6/85-vaccinated pullets as compared to moderate to high recovery rates of MG from ts-11 vaccines. Strain ts-11 appeared to colonize and persist in the trachea for the duration of the study, 105 days PV, following eyedrop inoculation, whereas strain 6/85 was apparently less effective at tracheal colonization following aerosol administration. These findings are consistent with a previous report (1) in that MG was isolated at a lower rate from tracheas of 6/85 vaccines than from ts-11-vaccinated chickens. It is possible that recovery rates for each vaccine strain could vary depending on the site cultured and may be influenced by the vaccination route.

Transmission of strain 6/85 from vaccines to commingled pullets or sentinel birds in adjacent pens was not observed during the trial. MG was recovered from ts-11-commingled pullets at low to moderate rates, whereas sentinel birds in adjacent pens were not positive by culture for MG. These findings indicate that strain ts-11 is transmissible from vaccines when birds are in direct contact with each other and/or share a common feeder and waterer, but transmission is unlikely with indirect contact.

ts-11-Vaccinated pullets had ELISA seropositive rates ranging from 10% at day 42 PV to 70% at day 105 PV. These data indicate that ELISA was moderately sensitive in its ability to detect MG antibodies in vaccinated pullets at

Fig. 3. Random amplified polymorphic DNA profiles of *Mycoplasma gallisepticum* (MG) strain ts-11 vaccine and MG isolates from strain ts-11 vaccinated and commingled pullets at various days postvaccination (PV). Lane 1, DNA size standards (Bio-Rad); lane 2, strain ts-11 vaccine; lane 3, negative control (reaction mixes without DNA); lane 4, isolate from vaccinated pullet 1R at day 105 PV; lane 5, isolate from vaccinated pullet 7R at day 105 PV; lane 6, isolate from vaccinated pullet 8R at day 84 PV; lane 7, isolate from vaccinated pullet 2R at day 21 PV; lane 8, isolate from vaccinated pullet 7R at day 21 PV; lane 9, isolate from commingled pullet 404Y at day 63 PV; lane 10, isolate from commingled pullet 405Y at day 42 PV; lane 11, isolate from commingled pullet 407Y at day 42 PV; lane 12, isolate from commingled pullet 410Y at day 63 PV. DNA sizes in kilobases are shown on the left.
times after 63 days PV. The highest MG culture positive rate in ts-11-commingled pullets was 40% at day 63 PV. However, the MG ELISA detected only one positive pullet, the same bird, at days 84 and 105 PV. It is possible that MG ELISA sensitivity for ts-11-vaccinated and -commingled pullets could increase with additional time PV. The SPA test detected MG seropositive rates in ts-11 vaccines only at days 42 (90%) and 105 (20%) PV; and in a commingled pullet at day 42 (10%) PV. Compared to ELISA, the SPA test yielded a higher seropositive rate in ts-11 vaccines earlier post-exposure (day 42 PV), but not later.

ELISA and SPA tests did not detect MG seropositive responses in strain 6/85 vaccines. HI tests detected no positive MG antibody response in either strain 6/85 or ts-11 vaccines. However, HI titers in the suspect range (1:40) were detected in a low to moderate percentage of ts-11-vaccinated pullets and a low percentage of ts-11-commingled pullets during the trial (data not shown). These results may reflect the relatively low sensitivity of MG HI tests compared to other tests, or antigen-dependent sensitivity that has been described for MG HI tests (3,11). Our results differ from those observed by Abd-el-Motelib et al. (1) in which ts-11- and 6/85-vaccinated chickens elicited a strong HI response at 4 wk PV. However, in their study chickens were vaccinated with 6/85 via the eyedrop route, which may account for the differences in 6/85 HI responses. In addition, these differences could in part be due to variability of HI test results among laboratories or the above-mentioned HI antigen-dependent sensitivity (3,11). Serology has been and continues to be the foundation of MG control programs; therefore, it is important to have more precise knowledge of the expected outcome from various types of poultry that may be exposed to live MG vaccines. Ideally, it would be useful to have strain-specific serologic tests that could be used to monitor and identify exposure to any MG vaccine strain.

RAPD analysis of MG vaccine strains 6/85 and ts-11 showed that the two vaccines were readily distinguishable from each other (Figs. 2, 3) and from F strain (data not shown) as demonstrated by their different DNA banding patterns. Additionally, there was consistency in the DNA banding patterns of MG isolates made in the ts-11 trial and vaccinal 6/85, and MG isolates made in the ts-11 trial and vaccinal ts-11. This was as expected and indicates that the biosecurity of each room was maintained, and the RAPD banding patterns of these vaccines were conserved for the duration of these trials. In the present study, we demonstrated that RAPD analysis can be used to compare DNA fingerprints of MG strains and distinguish vaccinal MG strains from each other. Our results are consistent with those of others (5,7) who concluded that the RAPD technique offers a fast and reproducible method for identifying MG strains.

REFERENCES


ACKNOWLEDGMENTS

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