ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 10 (5): 397-400, 2011
ISSN 1682-8356

Analysis of the Effect of Diluent for Rehydration of PoulVac® MycoF on Vaccination Seroconversion Results

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Abstract: Direct eye drop vaccination of poultry using live *Mycoplasma gallisepticum* vaccines provides the most efficient route of vaccination. The research reported in this study examines the effect of diluent used to rehydrate lyophilized *M. gallisepticum* vaccines on its ability to induce a measurable humoral immune response in the host. The results suggest that during the initial time period following vaccination, significant differences between the treatment groups are found. However, these differences between the diluents are unlikely to significantly impact the final outcome of vaccination when considered over the life of the host.

Key words: *Mycoplasma gallisepticum*, vaccination, diluent, rehydration

INTRODUCTION

Numerous routes are used to deliver vaccines to commercial poultry including injection, water delivery, spray and eye drop (Branton *et al*., 2005; Marangon and Busani, 2006). While any of these methods could be used with any vaccine, in practice only certain delivery methods work with individual vaccines either due to differences in vaccine viability, natural route of host infection, or economic factors (Marangon and Busani, 2006).

Only the last three methods mentioned above have been studied for delivery of live *Mycoplasma gallisepticum* (MG) vaccines (Branton *et al*., 2005). However, delivery through drinking water systems is not recommended due to the sensitivity of live mycoplasma vaccines to osmotic lysis as compared to other bacterial vaccines (Wieslander *et al*., 1992). Spray application is commonly used because it is less costly for mass application; however, it is also inefficient at applying the vaccine to the host (Branton *et al*., 2005). Direct vaccine delivery by eye drop appears to be the most efficient method if labor costs are sufficiently low or the increased protection provided by the high realized dose outweighs the increased cost of vaccine application (Bermudez and Stewart-Brown, 2003).

Previous research has shown that the diluent used to rehydrate the lyophilized MG vaccine can have a significant impact on vaccine survival, which also likely impacts the realized host immune response (Kleven, 1985; Leigh *et al*., 2008a; Leigh *et al*., 2008b). The choice of diluent is likely less critical for eye drop vaccination compared to spray vaccination due to the decreased dilution of the material contained in the lyophilized vaccine material. However, it is unknown what effect diluent choice will have on the outcome of MG eye drop vaccination. The purpose of this study is to determine how the diluent used to rehydrate the lyophilized MG vaccine effects the results of eye drop vaccination as measured by host humoral immune response.

MATERIALS AND METHODS

Animals: Day-old Hy-Line W-36 pullets used in this study were obtained from a commercial source. Pullets were housed on dry pine shavings as previously described (Burnham *et al*., 2002). Pullets were bled and swabbed at seven weeks of age and the serum was screened by Serum Plate Agglutination (SPA) assay and shown to be free of MG antibody. Swabs were inoculated in Frey's medium (Frey *et al*., 1968) and cultured as previously described (Branton *et al*., 1997).

Vaccination: At 13 weeks of age, pullets were moved to the biological isolation facility and vaccinated with PoulVac® MycoF (Fort Dodge Animal Health, Fort Dodge, IA). Three diluents were used, sterile distilled water, Sterile Diluent 28 (SD-28) which is supplied with PoulVac® ILT vaccine (Fort Dodge) and Opti-Vac® (Animal Science Products Inc., Nacogdoches, TX). The treatment groups were: 1) control, 2) PoulVac® MycoF in distilled water, 3) PoulVac® MycoF in SD 28 and 4) PoulVac® MycoF in Opti-Vac. All three diluents were at room temperature prior to vaccine rehydration and were added to identical 4°C vaccine vials from the same lot. Vaccine was rehydrated to a final concentration of one dose per 20 µl. Rehydrated vaccine was held on ice for up to one hour during the vaccination process. Pullets were vaccinated by dropping one dose (20 µl) in the right eye of each pullet. Immediately following vaccination, pullets were placed in biological isolation units (Branton and Simmons, 1992) at five pullets per isolation unit with four units per each treatment group.

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Serum testing: One pullet from each isolation unit was randomly selected and bled on days 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35 and 42 post-vaccination. Pullets were marked following bleeding so that the same pullet was not used for consecutive serum samples. The serum from the blood was tested by SPA assay using MG SPA antigens produced by both Intervet (Intervet Inc., Millsboro, DE) and Charles River (Charles River Laboratories International, Inc., Wilmington, MA). Following SPA analysis, serum samples were frozen and sent to the Mississippi State Veterinary Diagnostic Laboratory (Pearl, Mississippi) for ELISA (IDEXX Laboratories, Inc., Westbrook, ME) analysis. ELISA positive samples were further analyzed by the Mississippi State Veterinary Diagnostic Laboratory to determine their HI titers using HI antigen obtained from NVSL (National Veterinary Services Laboratories, Ames, IA).

Statistics: For statistical purposes, each isolation unit was considered to be a treatment group. Four isolation units were used for each treatment. Data was normalized by Log_{10} transformation prior to analysis. Data was analyzed using the One-Way ANOVA in SAS Analyst (SAS Institute Inc., Cary, NC) and results were further subdivided by serum collection date. Tukey's HSD test was used to determine the significance of differences with $p \leq 0.05$ considered significant.

RESULTS AND DISCUSSION

The results for eye-drop vaccination using any of the three diluents (distilled water, SD-28, or Opti-Vac) yielded similar results. By six days post vaccination, each of the three vaccinated groups showed positive SPA reactions, although only on day seven do the results become significantly different from the serum of the unvaccinated control group (Fig. 1). It wasn't until 14 days post vaccination, the next time the pullets were bled, that all three vaccine treatment groups were SPA positive and significantly different from the control group. At 14 days, all three groups were consistently giving SPA scores of 2 or 2+. These results suggest that the use of any of the three diluents is sufficient for the vaccine to provoke an equivalent host immune response. While there are slight differences in results at six and seven days post vaccination, the equivalent results obtained at 14 days post vaccination suggest that differences are unlikely to be seen if any of the three diluents are used under field conditions.

Positive ELISA reactions developed much later than the positive SPA response. Chickens administered vaccine rehydrated with distilled water exhibited positive ELISA results at 14 days post-vaccination, but it was only at 42 days post vaccination that each of the four replicates within the treatment group was both ELISA positive and significantly different from the control group (Fig. 2). Rehydration with SD-28 delayed a positive ELISA response until 28 days post-vaccination. Rehydration with Opti-Vac delayed a positive ELISA reaction until 35 days post-vaccination. At that time point, all replicates were MG ELISA positive; however, one of the Opti-Vac replicates was ELISA negative at 42 days post-vaccination. Statistical analysis of these results suggest that there is no difference among the three diluents used, although at 35 days post-vaccination, there is also no difference between Opti-Vac, distilled water and control treatment groups.

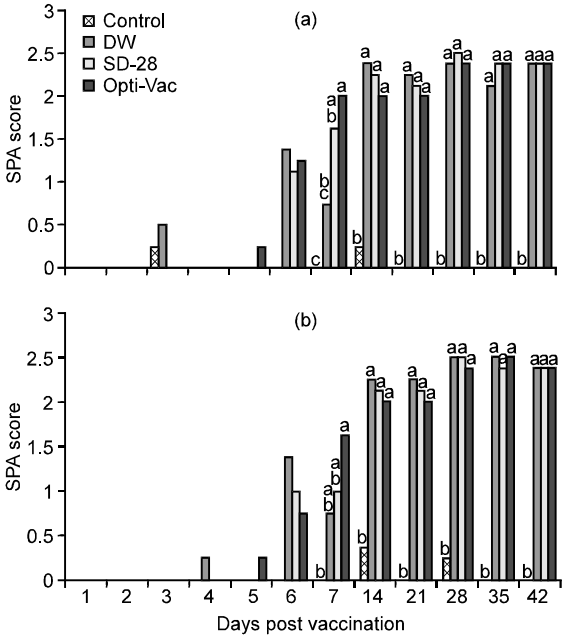

Fig. 1: Chickens SPA response. Y-axis is average SPA score and X-axis is time in Days post vaccination. (a) SPA response measured using Intervet MG antigen. (b) SPA response measured using Charles River MG antigen. Bars with differing low case letters are significantly different ($p \leq 0.05$)

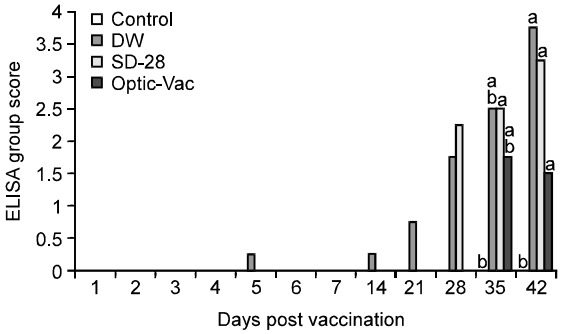

Fig. 2: Serum response as measured by ELISA. Y-axis is average ELISA Group score and X-axis is time in Days post vaccination. Bars with differing low case letters are significantly different ($p \leq 0.05$)

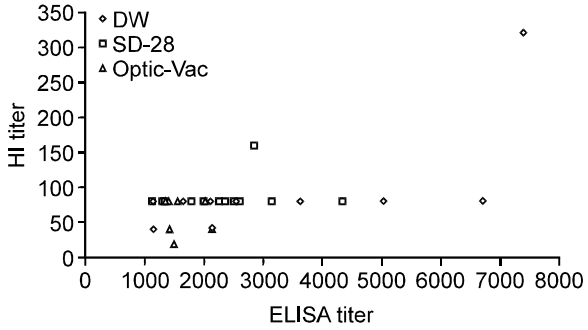

Fig. 3: Comparison of ELISA Group versus HI Titer. HI titer is represented on the Y-axis and ELISA Group is represented on the X-axis

HI analysis was performed on the serum samples that were positive for MG by ELISA. Both HI and ELISA predominantly detect chicken IgY antibodies. However, the results from these two assays do not correlate with each other (Fig. 3). ELISA groups were spread across the range, but HI titers tended to cluster at the low titer range. In at least one instance, an HI titer result was considered negative for a serum sample that was strongly positive both by SPA and ELISA. A possible explanation for this phenomenon could be due to differences between the F-strain MG used in the vaccine and the MG strain used by NVSL to create the MG HI antigen used by the Mississippi State Veterinary Diagnostic Laboratory. Research has shown that HI antigen from heterologous strains could lead to significantly lower HI titers as compared to the results obtained using a homologous MG strain (Dingfelder *et al*., 1991; Kleven *et al*., 1988; Roberts, 1969). This suggests caution is needed when selecting and interpreting presumptive and confirmative tests for MG infection as the results of the tests do not always accurately reflect each other and may lead to inaccurate conclusions, whether testing for response to vaccination or testing for MG infection by field strains.

While these results suggest a significant and equivalently rapid serological response based on the vaccine used with any of the three diluents, the measured humoral immune response does not infer the level of protection against later MG challenge or infection. Currently, only a limited amount of information is known about how the host immune response protects chickens from MG infection. However, there are a number of studies that suggest that the MG-induced serum humoral immune response does not correlate with protection from MG induced disease (Abd-el-Motelib and Kleven, 1993; Hildebrand *et al*., 1983; Lam *et al*., 1983; Ley, 2003; Noormohammadi *et al*., 2002; Soeripto *et al*., 1989; Talkington and Kleven, 1985). Although the serum humoral response does not appear to correlate with protection, both tissue localized antibodies and the cellular immune response have been implicated in protection from MG infection (Avakian and Ley, 1993; Ganapathy and Bradbury, 2003; Gaunson *et al*., 2000; Gaunson *et al*., 2006). This suggests that the IgY immune response, as measured by the ELISA and HI tests, may be irrelevant to actual protection from MG related disease, although limited protection from airsacculitis following transfer of high-titered MG antiserum has been demonstrated (Lin and Kleven, 1984). In general, the serum humoral immune response serves more as an indicator that a chickens has been exposed to MG rather than an indicator of the actual level of protection afforded by a vaccine.

The results of this study suggest that any of the three diluents tested will yield identical end results following host vaccination. There may be small variations in the time between vaccination and maximal immune response for all members of a treatment group. However, when considered in light of optimal vaccination time compared to the onset of lay, those differences are not great enough to change the outcome of eye drop vaccination at the 1X dose level. It is possible that there might be significant differences if either a dose of less than 1X per pullet were used or suboptimal conditions for vaccination exist that result in mortality of organisms within the live vaccine. Previous research on vaccine stabilizers suggests that the compounds contained in the lyophilized cell pellet for FVAX-MG vaccine, which is currently identical to PoulVac® MycoF used in this study, provide protection to the vaccine that ameliorates the decrease in viability over time following vaccine rehydration (Leigh *et al*., 2008a; Leigh *et al*., 2008b). These results were not seen with the other MG vaccine tested in those studies. Usage of different MG vaccines may result in variation of vaccination outcome when compared to the vaccine tested in this work.

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