Evaluation of duration of immunity after vaccination of swine with a single dose of Mycoplasma hyopneumoniae bacterin (M+Pac®)

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Introduction
Schering-Plough Animal Health Corporation (SPAHC) currently manufactures a Mycoplasma hyopneumoniae bacterin marketed under the trade name of M+Pac®. For primary immunization, the current label requires two 1 mL doses, given subcutaneously (SC) or intramuscularly (IM). The objective of this study was to evaluate the duration of immunity of M+Pac four months after vaccination with a single 2 mL dose, to support a label claim that the bacterin is effective when administered as a single dose for primary immunization. Efficacy was evaluated by challenging swine with a virulent M. hyopneumoniae culture 4 months after vaccination.

Methods
Twenty-one M. hyopneumoniae-negative swine were vaccinated IM with a single 2 mL dose at 6 weeks of age. Twenty nonvaccinated pigs were held as contact controls. All swine were bled monthly, and sera were tested for M. hyopneumoniae antibodies by both a monoclonal antibody-based blocking ELISA1 (DAKO Corp., Carpinteria, CA) and an indirect ELISA2 (HerdChek, IDEXX Laboratories, Inc., Westbrook, ME). Four months following vaccination, all swine were challenged transtracheally with 20 mL of a virulent, heterologous M. hyopneumoniae challenge culture obtained from Dr. Eileen Thacker’s Lab at the Veterinary Medical Research Institute (VMRI), Iowa State University. Four weeks after challenge, all swine were euthanatized by an AVMA-approved method and each pig’s lungs were examined for gross lesions typical of M. hyopneumoniae infection. The percent lung consolidation was evaluated by visual assessment and lung lobe scores were weighted, based on relative lobe weight as percent of total lung weight.3 Lung specimens from selected pigs were submitted to the Iowa Veterinary Diagnostic Laboratory for bacterial culture, histopathology, and M. hyopneumoniae immunohistochemistry. Total lung consolidation scores were evaluated using ANOVA procedures on arcsine-square root transformed data.

Results and discussion
Lung consolidation scores are summarized in Figures 1 and 2. At necropsy, all nonvaccinated controls (20/20) had gross lung consolidation, with a mean score of 7.08 ± 5.89% (median score 6.17%) and a range of 0.42 to 23.38%. The mean score in vaccinates was 2.40 ± 3.15% (median score 0.81%) with a range of 0 to 12.6%. Only 19% of the vaccinates, compared to 55% of the nonvaccinates, had consolidation involving > 5% of the lung. A majority (52% of vaccinates) had a lung score of < 1%, and 67% of the vaccinate scores were < 2%. The reduction of pneumonia in vaccinates was highly significant (P = 0.00035). Gross lesions were consistent with the cranial ventral consolidation typical of mycoplasma pneumonia. Lung specimens from 13 pigs were submitted to the Iowa Veterinary Diagnostic Laboratory for further analysis. All lung specimens showed microscopic lesions of peribronchial and peribronchiolar lymphoid hyperplasia and were positive for M. hyopneumoniae by immunohistochemistry. Although there were also microscopic lesions of purulent bronchopneumonia in 6/13 lungs, the only bacterial isolate was a low population of Actinobacillus suis isolated from one specimen.

All sera were tested for M. hyopneumoniae antibody titers using the DAKO test (Figure 3). All sera were negative at the prevaccination bleeding and all nonvaccinated swine remained seronegative during the prechallenge bleedings. At the 28-day post-vaccination bleeding, only 1/21 vaccinates was positive on the DAKO test. The number of positive vaccinates increased slightly at the subsequent pre-challenge bleedings, when 3/21 (day 56), 5/21 (day 84) and 4/21...
(day 119) were positive. These data indicate that although only 24% of vaccinates became positive for DAKO antibodies, antibodies were slow to develop and levels persisted in those pigs that did seroconvert following vaccination. Following challenge, all vaccinated pigs responded with a strong anamnestic response. The DAKO % inhibition rose from a pre-challenge mean titer of 33.5% to a postchallenge mean titer of 83.7%, and 19/21 vaccinates were positive for DAKO antibodies at necropsy. These results indicate that the single dose of M+Pac primed the immune response to subsequent challenge. A lower antibody response was evident in the nonvaccinates, with 7/20 nonvaccinates positive, 7/20 suspect, and 6/20 negative at the post-challenge bleeding. These results are consistent with previous studies that indicate that development of IgG antibodies may be delayed in swine exposed to *M. hyopneumoniae*.1,4

Sera from 3 bleeding dates were also tested for *M. hyopneumoniae* antibodies using the IDEXX ELISA test (Table 1). There was close agreement between the IDEXX and DAKO tests; however, the IDEXX test detected a higher number of positive antibody titers in vaccinated swine at each bleeding timepoint. Prechallenge sera from nonvaccinated swine were negative by both tests. However, the IDEXX test detected a higher percentage of positive nonvaccinated swine at the postchallenge bleeding (85% versus 35% for the DAKO test).

**Conclusions**

- This study demonstrates that a single dose of M+Pac is efficacious and protects swine from subsequent challenge 4 months after vaccination.
- When testing prechallenge sera, there was close agreement between the DAKO and IDEXX antibody tests. However, the IDEXX test detected a higher number of positive antibody titers in vaccinated swine and a higher percentage of positive antibody titers in sera from the nonvaccinated, challenged swine.
- Although the single dose did not elicit a strong antibody response to *M. hyopneumoniae*, all vaccinates developed an anamnestic response following challenge, indicating that the single dose of M+Pac has primed the immune response for subsequent challenge.
Table 1: Comparison of M. hyopneumoniae antibody titers using the DAKO and IDEXX ELISA tests.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Serological Results</th>
<th>2 Months Postvaccination</th>
<th>4 Months Postvaccination*</th>
<th>Post-challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-vaccinates</td>
<td>Mean Titer**</td>
<td>12.1%  0.00</td>
<td>14.0%  0.05</td>
<td>45.4%  0.75</td>
</tr>
<tr>
<td></td>
<td>% Positive</td>
<td>0%           0%</td>
<td>0%           0%</td>
<td>35%   85%</td>
</tr>
<tr>
<td></td>
<td>% Suspect</td>
<td>0%           0%</td>
<td>0%           0%</td>
<td>35%   10%</td>
</tr>
<tr>
<td></td>
<td>% Negative</td>
<td>100%         100%</td>
<td>100%         100%</td>
<td>30%   5%</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>Mean Titer</td>
<td>25.0%         0.29</td>
<td>33.5%         0.46</td>
<td>83.7% 1.36</td>
</tr>
<tr>
<td></td>
<td>% Positive</td>
<td>14%          24%</td>
<td>19%          33%</td>
<td>90%   95%</td>
</tr>
<tr>
<td></td>
<td>% Suspect</td>
<td>5%           14%</td>
<td>14%          10%</td>
<td>10%   5%</td>
</tr>
<tr>
<td></td>
<td>% Negative</td>
<td>81%          62%</td>
<td>67%          57%</td>
<td>0%    0%</td>
</tr>
</tbody>
</table>

*Prechallenge
**Key:
Mean DAKO titer = mean % inhibition; >50% is positive; between 35% and 50% is suspect; <50% is negative.
Mean IDEXX titer = mean S/P ration; >0.4 is positive; between 0.3 and 0.4 is suspect; <0.3 is negative.

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References