Evaluation of safety and efficacy of Mycoplasma hyopneumoniae bacterin (M+Pac™) when administered as single and two dose under field conditions

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Introduction
Mycoplasma hyopneumoniae causes enzootic pneumonia that primarily affects growing pigs. Although the disease is nonfatal, it causes substantial losses attributable to reduced growth performance, increased susceptibility to secondary bacterial pneumonia, and potentiation of PRRSV-induced pneumonia.1, 2 Both cell-mediated and humoral immune responses appear to be important in providing protection against infection.3

Schering-Plough Animal Health Corporation has been marketing M. hyopneumoniae bacterin (M+Pac®) as an aid to the control of pneumonia caused by M. hyopneumoniae infection. The vaccine is marketed as a single and two dose product. Several studies were conducted to demonstrate efficacy of M+Pac following experimental challenge (4-7). The present studies were conducted to evaluate the safety and efficacy of M+Pac under field conditions. These studies were conducted in a blinded manner.

Materials and methods
Animals
Two studies were conducted, one year apart, at two commercial farrow to finish farms in France. These studies were conducted following administration of a single dose or two doses of vaccine under field conditions. The single dose study was completed in April 2003 and the two dose study was completed in April 2004 at each of the two farms. For the one dose study, a total of 205 weaned pigs were vaccinated and additional 206 were used as placebo controls (Table 1). For the two dose study, a total of 190 pigs were used as vaccinees and additional 192 pigs were used as controls (Table 2).

<p>| Table 1: Number of pigs enrolled in single dose M+Pac study |
|-------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>97</td>
<td>108</td>
<td>205</td>
</tr>
<tr>
<td>Controls</td>
<td>98</td>
<td>108</td>
<td>206</td>
</tr>
<tr>
<td>Total</td>
<td><strong>195</strong></td>
<td><strong>216</strong></td>
<td><strong>411</strong></td>
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<p>| Table 2: Number of pigs enrolled the study in two dose M+Pac study |
|-------------------------|------------------|------------------|</p>
<table>
<thead>
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<th>Groups</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>90</td>
<td>100</td>
<td>190</td>
</tr>
<tr>
<td>Controls</td>
<td>91</td>
<td>101</td>
<td>192</td>
</tr>
<tr>
<td>Total</td>
<td><strong>181</strong></td>
<td><strong>201</strong></td>
<td><strong>382</strong></td>
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</table>

Vaccination
For single dose study, the pigs were vaccinated at 3-4 weeks of age. The pigs were vaccinated intramuscularly with 2 mL dose of vaccine. For two-dose study, first dose was given at 7-8 days of age and second dose was given at 14-28 days after initial vaccination. For two-dose study, the pigs were vaccinated intramuscularly with 1 mL dose of vaccine.

Antibody determination
Blood samples were collected at the time of initial vaccination and at various time points following vaccination until the time of slaughter. The sera were evaluated for the presence of antibodies by the monoclonal antibody based commercial test (DAKO A/S Glostrup, Denmark). The DAKO test is a competitive ELISA and the values are expressed as percent inhibition. The values > 50% inhibition are considered positive for antibody response.
Clinical observation
The pigs were observed 4-6 hours after vaccination and daily for 14 days after vaccination and then the health status was recorded on weekly basis until slaughter.

Average daily weight gain
The pigs were weighed prior to vaccination, at the end of post-weaning period, and just before slaughter.

Lung consolidation
The pigs were sent to slaughter at approximately 6 months of age and the lungs were examined for typical *M. hyopneumoniae* lesions and the percent consolidation was calculated as described by Christensen.8

Statistical analysis
Percent lung consolidation and antibody response were analyzed by Wilcoxon Rank Sum Test. Average daily weight gain was analyzed by analysis of variance procedures.

Results and discussion
Antibody response
The antibody response at each site for single dose study are shown in Figures 1 and 2. The pigs in both vaccinate and control groups were seronegative at the time of vaccination. The vaccinated pigs did not show any increase in antibody response until slaughter time. These results are consistent with previous studies showing that pigs administered with a single dose of vaccine do not show increases in antibody response. The pigs in both groups showed elevated antibody response at slaughter which suggests natural exposure to disease. The antibody response in vaccinated group at slaughter in both sites was significantly higher than in controls \( P \leq 0.0062 \). These results suggests an anamnestic response in vaccinated pigs due to natural exposure.

The antibody response at each site for two-dose study are shown in Figure 3 and 4. The pigs in both vaccinate and control group were sero-positive at the time of vaccination. These titers are presumed to be due to maternal antibodies still present at one week of age. The passive antibody titer in control pigs dropped to negative level a couple weeks later and remained negative until the time of slaughter. The antibody response in vaccinated pigs increased significantly following booster vaccination and the titer held constant until the time of slaughter. The antibody response in vaccinated pigs was significantly higher \( P \leq 0.0026 \) at all time points compared to controls in sera samples collected following booster vaccination. The pigs in the control group showed elevated antibody response at slaughter indicating natural exposure some time before slaughter.

Lung consolidation
Percent lung consolidation at slaughter in both sites for single dose are shown in Figure 5. The vaccinated pigs at site 1 had a median lesion score of 1.6 and the controls had a median score of 12.4. The lesion score in vaccines was significantly lower \( P = 0.0001 \) than controls. The vaccinated pigs at site 2 had a median lesion score of 4.9 and the controls had a median score of 15.3. The lesion score in vaccines was significantly
Percent lung consolidation at slaughter in both sites for two-dose are shown in Figure 6. The vaccinated pigs at site 1 had a median lesion score of 0.9 and the controls had a median score of 3.6. The lesion score in vaccines was significantly lower ($P = 0.0062$) than controls. The vaccinated pigs at site 2 had a median lesion score of 1.0 and the controls had a median score of 4.9. The lesion score in vaccinates was significantly lower ($P = 0.0018$) than controls. The vaccine demonstrated 75% efficacy at site 1 and 79% efficacy at site 2, compared to the controls, in reducing lung consolidation.

Average daily weight gain

Average daily weight gain for pigs in single dose study are shown in Figure 7. The vaccinated pigs at site 1 gained an average of 0.809 kg/day and the pigs in control group gained 0.758 kg/day. The average daily weight gain in vaccinated pigs was significantly higher ($P = 0.0146$) than the average weight gain in control group. The vaccinated pigs at site 2 gained an average of 0.805 kg/day and the pigs in control group gained 0.768 kg/day. The average daily weight gain in vaccinated pigs was numerically higher and approaching significant level ($P = 0.0521$) compared to average weight gain in control group.

There was no overall significant difference in weight gain in the two-dose study. However, heavier pigs (> 1 Std dev of mean) at the time of initial vaccination in lower ($P = 0.0007$) than controls. The vaccine demonstrated 87% efficacy at site 1 and 68% efficacy at site 2, compared to the controls, in reducing lung consolidation.
M+Pac group gained significantly higher ($P = 0.0487$) average daily weight gain compared to equally heavier pigs in control group. Further, the pigs in the two-dose study had higher concurrent infections and higher incidence of diarrhea (>6.5%) compared to single dose study (<1% incidence of diarrhea). Therefore, the failure to show significant differences in ADG for vaccinates compared to controls in the second study may be due to lower *M. hyopneumoniae* challenge (based on lower lung score in controls) or the effect of concurrent disease.

**Safety**

None of the pigs in the single dose study showed any systemic reaction or injection site reaction that is attributable to the vaccine.

None of the pigs in the two-dose study showed injection site reaction. Eleven pigs (10 vaccinates and 1 control) showed mild, transient clinical signs after first vaccination which were not considered serious by the study investigators.

**Conclusions**

- M+Pac provided significant protection against lung lesions through slaughter when used as a single or two dose regimen under field condition.
- M+Pac can overcome maternal antibody interference under field condition.
- M+Pac is found safe when administered as one dose or two dose under field conditions.
- M+Pac demonstrated significant average daily weight gain under field conditions.

**References**