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EFFECT OF SOW VACCINATION AGAINST MYCOPLASMA HYOPNEUMONIAE ON SOW AND PIGLET COLONIZATION AND SEROCONVERSION, AND PIG LUNG LESIONS AT SLAUGHTER


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Abstract

The objectives of the present study were to compare *Mycoplasma hyopneumoniae* (Mh) colonization and serologic status on Mh vaccinated and non-vaccinated sows and to assess the effect of sow vaccination on colonization and serologic status of their piglets at weaning as well as presence of Enzootic Pneumonia (EP) lung lesions at slaughter.

Fifty sows (25 vaccinated and 25 unvaccinated) as well as 5 of their piglets were included in the study. Blood samples and nasal swabs from sows at 7 weeks pre-farrowing and 1 week post-farrowing and from piglets at 3-4 weeks of age were taken. Nasal swabs and sera were tested by a nested Polymerase Chain Reaction (nPCR) and by an enzyme-linked immunosorbent assay (ELISA) test to detect Mh DNA and antibodies to the pathogen, respectively. Finally, at 23 weeks of age, pigs were sent to the slaughter where the extension of EP-compatible gross lesions was assessed.

Vaccination with two doses of Mh vaccine resulted in a significantly higher (p<0.05) percentage of seropositive sows than in the non-vaccinated group at 1 week post-farrowing. On the contrary, no statistical significant differences were found in the number of nasal nPCR positive sows among different treatments (p>0.05). At 3-4 weeks of age, a significantly higher percentage (p<0.001) of seropositive piglets came from vaccinated than from non-vaccinated sows. Although the number of Mh infected piglets coming from non-vaccinated sows was higher than the one from vaccinated sows, the difference was not statistically significant (p>0.05). Overall, piglets from vaccinated sows had a significant lower (p<0.05) mean of EP-compatible lung lesions (1.83±2.8) than piglets from non-vaccinated sows (3.02±3.6). Under the conditions described in this study, sow vaccination did not affect sow or piglet colonization but increased the percentage of seropositive sows and piglets at weaning and reduced significantly the mean EP-compatible lung lesion scoring at slaughter.
1. Introduction

*Mycoplasma hyopneumoniae* (Mh) is the causative agent of enzootic pneumonia (EP), a chronic respiratory disease that affects mainly growing and finishing pigs (Thacker, 2006).

Vaccination strategies against Mh vary depending on the type of herd, production system, Mh infection dynamics and number of vaccine doses applied. Although efficacy of commercial vaccines in reducing EP lung lesions has been extensively proven, protection conferred seems to be incomplete since vaccinated animals can be also infected (Haesebrouck et al., 2004). Another putative strategy to ameliorate the effects of Mh infection is to reduce the vertical transmission of the pathogen and to elicit and to transfer strong colostrum derived immunity to the piglets by means of sow vaccination. Under field conditions, Mh sow vaccination is not frequently included in vaccination strategies, with the exception of gilts in acclimatisation programmes (Bargen, 2004). So far, sow vaccination has been applied to assess its effects on serologic and colonization status in vaccinated (Grosse Beilage and Schreiber, 2005; Jayappa et al., 2001; Martelli et al., 2006) and non-vaccinated (Calsamiglia and Pijoan, 2000; Ruiz et al., 2003) weaning piglets. On the other hand, Diaz et al., (2004) evaluated the effect of sow vaccination on lung lesion development in Mh vaccinated piglets. Nevertheless, the effect of Mh vaccine-induced passive immunity on EP lung lesions development in non-vaccinated slaughtered pigs has not been described.
Therefore, the objectives of the present study were 1) to compare Mh colonization and serologic status on Mh vaccinated and non-vaccinated sows and 2) to assess the effect of sow vaccination on colonization and serologic status of their piglets at weaning as well as presence of EP lung lesions at slaughter.

2. Materials and methods

2.1. Farm

A 150 sow, farrow-to-finish farm with EP–related respiratory problems in finishing pigs was selected to carry out this study. No Mh-vaccination was used in the farm before the starting of the present field study. Mh infection was confirmed one month before the initiation of the study by nested PCR (nPCR) in nurseries in 55 3-week-old piglets (7 out of 55 [12.8%] were positive by nPCR in tonsillar swabs), in 20 slaughtered animals by serology (17 out of 20 [85%] were seropositive to Mh) and by EP-compatible lung lesion scoring (mean gross lung lesion score of 6.5 based on the classification by Hannan et al., [1982]).

2.2. Study design

Fifty sows were selected 7 weeks pre-farrowing; nasal swab and blood were taken from all of them. Parity distribution among these 50 sows were 2-4 (n=21, 42%), 5-7 (n=24, 48%) and more than 7 (n=5, 10%). At 5 weeks pre-farrowing, sows were divided into two groups (vaccinated and non-vaccinated) taking into account the Mh ELISA and nPCR results of the samples taken at 7 weeks pre-farrowing (Table 1). At 5 and 3 weeks pre-farrowing, 25 sows received 2 ml of an intramuscular injection of MYPRAVAC-SUIS® (HIPRA, Spain) and the rest 25 sows received a 2 ml intramuscular injection of PBS. This vaccine is based on a suspension of inactivated Mh strain J with a carbomer/levamisol adjuvant. At 1 week post-farrowing, nasal swab and
blood were taken from each sow and a mean of 5 piglets per sow were randomly
selected and ear-tagged. From the 246 piglets included in the study, 122 and 124 came
from vaccinated and non-vaccinated sows, respectively. Animals were weaned at 17 to
23 days of age and transferred to the nursery units, where litters from different sows
were mixed. At 3-4 weeks of age, nasal swabs and blood were taken from all these
piglets. At 9 weeks of age, pigs were moved to the growing-finishing units and they
were distributed taking into account the body condition, following the farmer criterion.
Finally, at 23 weeks of age, pigs were sent to the slaughter and extension of EP-
compatible gross lesions (craneo-ventral pulmonary consolidation) was assessed as
previously described (Hannan et al., 1982).

Treatments, housing, husbandry and slaughtering conditions conformed to the
European Union Guidelines and Good Clinical Practices.

2.3. DNA extraction and nPCR procedures

DNA extraction from nasal swabs and Mh nPCR were performed as previously
described (Sibila et al., 2004). Mh antibodies in serum samples were detected using a
monoclonal blocking ELISA test (CIVTEST suis® MYCOPLASMA
HYOPNEUMONIAE, HIPRA. Girona, Spain) following manufacturer’s instructions.
The inhibition percentage (IP) was calculated taking into account the optical densities
(OD) of each sample as well as the one of the negative control (negative value). ELISA
results interpretation, based on IP values, was as follows: IP < 50%, negative; IP > 50%,
positive.

2.4. Statistical Analyses

Bivariate analyses using contingency tables (Chi-square statistics or Fisher’s
exact test for 2x2 tables) were used to compare the following parameters among
different treatment groups: 1) number of Mh seropositive sows at 7 weeks pre- and 1
week post-farrowing; 2) number of nPCR positive sows at nasal cavity at 7 weeks pre- and 1 week post-farrowing; 3) number of Mh seropositive piglets at 3-4 weeks of age; and 4) number of nPCR positive piglets at 3-4 weeks of age. In order to study the influence of parity number on several parameters, sows were divided into previously described groups: 2 to 4, 5 to 7 and higher than 7 (Calsamiglia and Pijoan, 2000). Contingency tables were also used to analyze the effect of parity number on sow and piglet serologic and colonization status. Non-parametric statistical analysis (Kruskal-Wallis statistic) was used to test differences in gross lung lesion scoring among animals from different treatments groups. On the other hand, Wilcoxon statistics were used to assess the relationship between gross lung lesion scoring at slaughter with nPCR positive and negative pigs at the weaning age. Statistical analyses were performed with the SAS system for Windows version 8.0 (SAS Institute Inc, Cary, North Carolina, USA). Statistical significance level was set at $\alpha=0.05$.

Vaccine efficacy in reducing lung lesions measured at slaughter was calculated using group median lung scores (Jones et al., 2005), as follows: Vaccine efficacy = 
\[ \frac{\text{median non-vaccinated group score} - \text{median vaccinated group score}}{\text{median non-vaccinated group score}} \times 100. \]

3. Results and Discussion

Vaccination with two doses of vaccine resulted in a significantly higher ($p<0.05$) percentage of seropositive sows at 1 week post-farrowing than in the non-vaccinated group (Table 1). While in the non-vaccinated group, percentage of seropositive sows varied from 24% (6 out of 25) pre-treatment to 36% (9 out of 25) post-treatment administration ($p>0.05$), the figures changed from 28% (7 out of 25) to 96% (22 out of 23; serum from two sows was lacking) for vaccinated animals ($p<0.05$). Percentage of seropositive vaccinated sows at 1 week post-farrowing was similar to the ones obtained
in previously reported studies where sows were double vaccinated at 5 and 3 weeks pre-
farrowing (Ruiz et al., 2003) or single vaccinated at 2 weeks pre-farrowing (Martelli et al., 2006). No statistical significant differences between vaccinated and non-vaccinated groups were found in the number of nasal nPCR positive sows at 1 week post-farrowing (p=0.14). However, it is noteworthy that the percentage of nPCR positive sows at 7 weeks pre-farrowing and at 1 week post-farrowing showed a more marked decrease in the vaccinated sows group (from 28% to 0%) compared to that of the non-vaccinated sows (from 12% to 8%).

At 3-4 weeks of age, a significantly higher percentage (p<0.001) of seropositive piglets came from vaccinated sows (60 out of 118 [51%]) compared to non-vaccinated sows (3 out of 119 [3%]) (Table 2). Martelli et al. (2006) and Ruiz et al. (2003), reported also higher percentage of seropositive piglets coming from vaccinated (80% and 81-82%, respectively) than from non-vaccinated (0% and 5-21%, respectively) sows. On the other hand, Kristensen et al. (2004) and Grosse Beilage and Shreiber (2005) described a significantly higher level of Mh antibodies in pigs from vaccinated sows compared to control ones up to 3 and 4 weeks of age, respectively. Although, in the present study there were 37 out of 58 (64%) and 75 out 116 (64.5%) seronegative piglets that came from seropositive vaccinated and non-vaccinated sows, respectively. This observation may be due to the half-life of maternal antibodies to Mh, calculated as 15.8 days (Morris et al., 1994), and therefore at 3-4 weeks of age they might be very low or have already disappeared. On the other hand there were 23 seropositive piglets (20 and 3 from vaccinated and non-vaccinated sows, respectively) that came from seronegative sows. The latter ones may be explained by cross-fostering practice, although this practice was seldom performed in this farm.
On the other hand, only 8 out of 244 (3.3 %; nasal swab from two piglets was lacking) piglets were Mh nPCR positive at 3-4 weeks of age. This low percentage of Mh detection at nasal cavity in nursery pigs is similar to the one reported by another field study in a farrow-to-finish operation (Sibila et al., 2007a) Although number of piglets infected by Mh coming from non-vaccinated sows was higher than the one from vaccinated sows (5 versus 3), the difference was not statistically significant (p>0.05). These low numbers may have been the cause of no statistical significant differences found between piglets from vaccinated versus non-vaccinated sows. However, this low percentage of Mh infected pigs may be explained by a low infectious pressure present in the farm at that moment or by a, less probably, limited sensitivity of the nPCR used. It is generally believed that vaccination against Mh does not prevent from infection (Thacker, 2006) and, therefore, it has not a clear effect on sow or piglet colonization (Calsamiglia and Pijoan, 2000). More recently, however, it has been described that piglet vaccination may help in reducing the prevalence of Mh infected animals (Baccaro et al., 2005; Ruiz et al., 2003; Sibila et al., 2007b). Therefore, the present study does no help in elucidating the effect of Mh vaccination on sow and piglet colonization.

It is worthy to remark that 6 (75%) of these 8 nPCR positive piglets were seronegative, supporting the idea that passive immunity may decrease the percentage of infected piglets (Ruiz et al., 2003; Sibila et al., 2007a).

Due to loss of the ear-tag at the slaughterhouse, lung lesion scoring was only assessed on 198 (100 from vaccinated and 98 from non-vaccinated sows) out of 246 initially included pigs. Seventy six out of 198 (38,3%) did not show any EP-compatible lung lesion (Table 3). The highest lung lesion score observed was 16 (from a maximum of 35) in one piglet in each treatment. Overall, piglets from vaccinated sows had a significant lower (p<0.05) mean of EP-compatible lung lesions (1.83±2.8) than piglets.
from non-vaccinated sows (3.02±3.6). Vaccine efficacy in reducing lung lesions score was 50%. Díaz et al. (2004), reported a low incidence and grade of lung lesions in vaccinated piglets coming from vaccinated sows compared with the ones from non-vaccinated sows. In this latter study the effect of sow vaccination on their non-vaccinated piglets was not truly assessed since lung lesion development was compared between double Mh vaccination (sow and piglets) versus piglet vaccination at different ages. Therefore and as far as the authors’ knowledge, this is the first description of the effect of Mh sow vaccination in reducing EP-compatible lung lesions in their non-vaccinated piglets at slaughter. It is known that Mh vaccine effect are greater with higher Mh infectious pressure (Maes et al., 1999); and therefore, the relatively low levels of infection and of EP lung lesions observed in the present study might have prevented to see a more significant effect of the vaccine used.

An interesting point to remark was the significant relationship (p<0.05) between positivity by nPCR at 3-4 weeks of age and lung lesion scoring at slaughter (Fano et al., 2006a; Sibila et al., 2007b). Specifically, piglets infected by Mh at 3-4 weeks of age had greater mean lung lesion score (7.00±5.2) than non-infected piglets (2.28±3.0). Therefore, our results further support that the detection of Mh at nasal cavity of weaning pigs can be used as an indicator of EP-compatible lung lesions at slaughter-age (Fano et al., 2006b).

In summary, under the conditions described in this study, sow vaccination did not affect piglet colonization but increased the percentage of seropositive piglets at weaning and reduced significantly the mean EP-compatible lung lesion scoring at slaughter.
Acknowledgements

The authors are grateful to Eva Huerta and Merche Mora for technical assistance.

References


hyopneumoniae in pig herds with an all-in/all-out production system. Vaccine 17, 1024-1034.


**Table 1:** Number (and percentage) of positive sows by ELISA and nPCR at 7 weeks pre- and 1 week post-farrowing in both treatments (Mh vaccinated and non-vaccinated) and their parity distribution. Different superscripts within a column mean statistical significant differences.

<table>
<thead>
<tr>
<th>Sows</th>
<th>7 weeks pre-farrowing</th>
<th>1 week post-farrowing</th>
<th>Parity distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA + (%)</td>
<td>nPCR + (%)</td>
<td>ELISA + (%)</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>7 (28)</td>
<td>7 (28)</td>
<td>22 (96)×</td>
</tr>
<tr>
<td>Non-vaccinated</td>
<td>6 (24)</td>
<td>3 (12)</td>
<td>9 (36)×</td>
</tr>
<tr>
<td>Total</td>
<td>13 (26)</td>
<td>10 (20)</td>
<td>31 (65)</td>
</tr>
</tbody>
</table>
### Table 2: ELISA and nPCR results of piglets at 3-4 weeks of age taking into account the treatment received by the sows (Mh vaccinated and non-vaccinated) as well as their serologic and infectious status at 1 week post-farrowing.

<table>
<thead>
<tr>
<th>Piglets (3-4 weeks of age)</th>
<th>ELISA</th>
<th>nPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Vaccinated</strong> (n=23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n=22)</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>Negative (n=1)</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td><strong>Non-vaccinated</strong> (n=25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n=9)</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>Negative (n=16)</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>116</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sows (1 week post-farrowing)</th>
<th>ELISA</th>
<th>nPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Vaccinated</strong> (n=25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n=0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative (n=25)</td>
<td>63</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>59</td>
</tr>
<tr>
<td><strong>Non-vaccinated</strong> (n=25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n=2)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Negative (n=23)</td>
<td>3</td>
<td>106</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>116</td>
</tr>
</tbody>
</table>
Table 3: EP lung lesion scoring at slaughter in pigs from vaccinated and non-vaccinated sows.

<table>
<thead>
<tr>
<th>Lung lesions scoring</th>
<th>Piglets from vaccinated sows</th>
<th>Piglets from non-vaccinated sows</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48</td>
<td>28</td>
<td>76 (38)</td>
</tr>
<tr>
<td>1-5</td>
<td>41</td>
<td>47</td>
<td>88 (44)</td>
</tr>
<tr>
<td>6-10</td>
<td>7</td>
<td>19</td>
<td>26 (13)</td>
</tr>
<tr>
<td>11-15</td>
<td>3</td>
<td>3</td>
<td>6 (3)</td>
</tr>
<tr>
<td>16-20</td>
<td>1</td>
<td>1</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>98</td>
<td>198 (100)</td>
</tr>
</tbody>
</table>