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Effect of different adjuvants on PCV2-associated lesions

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Summary and Implications
Ninety, 12-14 day old pigs were randomly assigned to five groups. Group 1 (n=19) pigs were vaccinated with a Mycoplasma hyopneumoniae (M. hyopneumoniae) vaccine with an oil-in-water adjuvant (RespiSure®; Pfizer Animal Health, Inc.). Group 2 (n=17) pigs were vaccinated with a commercial M. hyopneumoniae vaccine with an aqueous adjuvant (Carbopol) (Suvaxyn® Respifend® MH; Fort Dodge Animal Health, Inc.). Group 3 (n=18) pigs were vaccinated using an oil-in-water adjuvanted vaccine containing the same amount and type of M. hyopneumoniae antigen as in group 2. Group 4 (n=18) pigs were vaccinated using an aluminum hydroxide adjuvanted vaccine containing the same amount and type of M. hyopneumoniae antigen as in group 2. Group 5 (n=18) pigs served as the controls and were sham-vaccinated with saline. Pigs were injected with 2 mL of one of the four M. hyopneumoniae vaccines at four and again at six weeks of age. PCV2 was inoculated intranasally on the day of the second vaccination at 6 weeks of age. Half of the pigs were necropsied at 21 days post inoculation (DPI). The remaining pigs were necropsied at 35 DPI.

There were no differences among groups in clinical disease scores. At 21 DPI all vaccinated groups had significantly (p<0.05) more severe lymphoid depletion than the saline injected group. At 35 DPI group 1 pigs had significantly (p<0.05) higher amounts of PCV2 DNA in serum than pigs in groups 2, 4, and 5 as determined by quantitative real-time PCR. There was a significant (p<0.05) increase in the severity of lymphoid depletion in the lymph nodes, tonsil, and spleen in groups 1 and 3 compared to groups 2, 4, and 5. Group 3 had significantly (p<0.05) higher amounts of PCV2 antigen within lymph nodes, tonsil, and spleen compared to groups 2, 4 and 5. The results confirm that all adjuvants tested enhanced PCV2-induced lesions and oil-in-water products used in this study had a more severe effect.

Introduction
PCV2 is associated with postweaning multisystemic wasting syndrome (PMWS) and has quickly become a major global problem in the swine industry. Recent experiments in Europe and by our group in the U.S. have confirmed that vaccination of pigs with commercially available M. hyopneumoniae bacterins significantly increases the length of PCV2 viremia, the amount of PCV2 in serum and lymphoid tissues, and the severity of PCV2-associated lymphoid depletion.

The objectives of this study were to determine if commercially available adjuvants (as opposed to the antigen) enhance PCV2-associated lesions and if there is a difference among adjuvants in this enhancement. If differences are present producers may need to consider this in vaccine selection.

Materials and Methods
Ninety, 12-14 days old pigs were randomly assigned to five groups of 17-19 pigs each. Groups 1-4 pigs were vaccinated with 2 mL of one of four M. hyopneumoniae bacterins at four and again at six weeks of age (Table 1). To exclude a potential effect of the M. hyopneumoniae antigen, three of the vaccines (groups 2-4) used in this model contained the same type and amount of M. hyopneumoniae antigen and varied only in the kind of adjuvant used.

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjuvant type</th>
<th>Mycoplasma antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oil-in-water type 1</td>
<td>Type A</td>
</tr>
<tr>
<td>2</td>
<td>Carbopol</td>
<td>Type B</td>
</tr>
<tr>
<td>3</td>
<td>Oil-in-water type 2</td>
<td>Type B</td>
</tr>
<tr>
<td>4</td>
<td>Aluminum hydroxide</td>
<td>Type B</td>
</tr>
<tr>
<td>5</td>
<td>Control (Saline)</td>
<td>Control (Saline)</td>
</tr>
</tbody>
</table>

Vaccines used in group 1 (RespiSure®, Pfizer Animal Health) and group 2 (Suvaxyn® Respifend® MH, Fort Dodge Animal Health) are commercially available. The vaccines in groups 3 and 4 are experimental. All pigs were inoculated intranasally with PCV2 isolate ISU-40895 on the day of the second vaccination at 6 weeks of age.

Blood was collected weekly and analyzed for the presence of PCV2-specific antibodies by an ELISA and to assess the amount of PCV2 by quantitative real-time PCR. Necropsies were performed on half of the pigs at 21 and half at 35 DPI. Selected tissue samples (lung, lymph nodes, spleen, liver, kidney, tonsil, heart) were placed in 10% neutral buffered formalin and routinely processed for histopathological examination. Immunohistochemistry (IHC) for PCV2 was done on paraffin embedded sections of lymph nodes, spleen and tonsil.

Analysis of variance (ANOVA) was used to check for differences among groups. If the ANOVA was significant (p<0.05) pairwise Wilcoxon tests were performed to identify the groups that were different.

Results and Discussion
Clinical scores and average daily weight gain were similar among groups. Significant differences among groups were not evident for severity of gross lesions. All vaccinated groups had significantly (p<0.05) more severe lymphoid depletion than the controls at 21 DPI.

At 35 DPI, there was significantly (p<0.05) more severe lymphoid depletion and granulomatous inflammation in the lymph nodes, spleen, and tonsil in group 1 compared to groups 2 and 4. Group 3 had
significantly (p<0.05) higher amounts of PCV2 antigen in lymph nodes, tonsil, and spleen compared to group 2 and 4. At 35 DPI, group 1 pigs had significantly (p<0.05) higher amounts of PCV2 DNA in serum than pigs in groups 2, 4, and 5.

This work suggests that at the early stages of PCV2 infection (21 DPI), all the adjuvants tested (oil-in-water, carbopol, aluminum hydroxide) increased the severity of lymphoid depletion associated with PCV2. In the later stages of infection (35 DPI), the oil-in-water adjuvants increased the severity of PCV2-associated lesions, viremia length, and amount of PCV2 in serum and tissue compared to carbopol and aluminum hydroxide products.

Producers with recurrent PCV2-associated disease may want to consider switching to the carbopol or aluminum-hydroxide adjuvanted products, discontinuing use of vaccines on PCV2-infected pigs, or changing the timing of vaccine administration. However, producers and practitioners must always consider the risk and benefit from elimination of vaccination and decrease control of coinfection.

Acknowledgments
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