A comparative co-infection challenge study was conducted in 2005 to assess previous reports that *Mycoplasma hyopneumoniae* bacterins with oil-based adjuvants may significantly increase the severity of lesions induced by porcine circovirus type 2 (PCV2) compared to placebo controls or bacterins with aqueous adjuvants.\(^1\)\(^-\)\(^4\) Clinical effects, macroscopic and microscopic lesions at necropsy examination, and growth performance were measured in pigs challenged with *M. hyopneumoniae* and PCV2 following two vaccinations with either a placebo or one of three commercial *M. hyopneumoniae* bacterins, two with oil-based adjuvants and one with an aqueous adjuvant. Compared to the placebo controls, the vaccine groups had no significant (P>0.05) differences in PCV2 levels in serum, severity of PCV2 macroscopic and microscopic lesions, increase in sort loss, or increase in number of pigs developing postweaning multisystemic wasting syndrome (PMWS). To the contrary, compared to the placebo controls, pigs vaccinated with all three *M. hyopneumoniae* bacterins had significantly lower lung lesion scores at the Day 63 necropsy, higher body weights and higher average daily gain (ADG) at the end of the study (P≤0.05). Differences among the three bacterins were also apparent. Compared to Bacterin A, RespiSure™-vaccinated pigs had significantly (P≤0.05) lower percent lung lesions and levels of *M. hyopneumoniae* in bronchoalveolar lavage (BAL) samples at the Day 63 necropsy and significantly fewer days with clinical signs. Compared to Bacterin B (with aqueous adjuvant), RespiSure pigs had significantly (P≤0.05) lower levels of *M. hyopneumoniae* in Day 63 BAL samples and higher *M. hyopneumoniae* antibody titers. At the Day 77 necropsy, only the RespiSure vaccinates had significantly (P≤0.05) lower lung lesions scores than the placebo controls. Overall, the results do not support previous reports that *M. hyopneumoniae* vaccination enhances losses associated with PCV2 infection. Rather, the results support the conclusion that control of *M. hyopneumoniae* infection by vaccination is an important health management tool in pig populations where PCV2 and *M. hyopneumoniae* are circulating.

Analysis of estimated market value conducted at the end of the study showed that although the overall treatment effect (P = 0.0603) was above the significance level tested, pigs vaccinated with RespiSure had the highest overall market value, with an advantage of $9.63 per pig compared to the placebo control, $3.80 compared to the competitor’s bacterin with an oil-based adjuvant, and $2.50 compared to the competitor’s bacterin with an aqueous-based adjuvant.
Several journals have published reports suggesting that vaccination with *M. hyopneumoniae* bacterins formulated with oil-based adjuvants may significantly increase PCV2 replication and severity of PCV2-induced lymphoid depletion compared to placebo controls and bacterins formulated with aqueous adjuvants.²⁻⁴ The study presented here was conducted to measure and compare clinical signs, macroscopic and microscopic lesions, and production losses or gains associated with use of three different commercially available *M. hyopneumoniae* bacterins in swine co-infected with *M. hyopneumoniae* and PCV2.

**Study Overview: Co-infection Model, Assessments, and Analysis**

**Study Design**

A total of 296 apparently healthy 3-week-old weaned barrows were enrolled in the study. The source farm had no history of vaccination or disease due to *M. hyopneumoniae* or PCV2. All pigs included in the analysis were negative for antibodies to *M. hyopneumoniae* at the time of first vaccination and negative for antibodies to PCV2 at the time of PCV2 challenge. The study site was a commercial fan-ventilated swine building with double-sided curtains and slatted floors.

The study was a generalized block design with the blocking factor based upon body weight and pen location (Table 1). The vaccines were coded and the individuals performing animal observations, in vitro assays, and necropsy observations and scoring were masked as to the assignment of animal to treatment.

**Challenge Materials**

*M. hyopneumoniae* lung homogenate (isolate 232) was provided by the laboratory of Eileen Thacker, DVM, PhD, Iowa State University.²⁰ The PCV2 challenge material was provided by Dr. Shan Yu in Dr. Thacker’s laboratory. The challenge inoculum was PK-15 cell culture passaged live virus prepared from PCV2 40985 molecular clone.²¹

**Assessments**

During the course of the study, the general health status of the pigs was monitored daily. Blood samples were taken on Study Days -2, 13, 34, 48, 62 (pigs allotted for necropsies), 76, 100, and 131. Serum samples were tested as follows:

- Antibodies to *M. hyopneumoniae* and PCV2. Enzyme-linked immunosorbent assay (ELISA) tests were run in Dr. E. Thacker’s laboratory.²²,²³
- Real-Time Polymerase Chain Reaction (RT-PCR). The PCV2 RT-PCR was run on the Day 48, 62, and 76 sera from pigs scheduled for necropsy by Dr. Tanja Opriessnig, Iowa State University. The results were expressed as PCV2 genome copy number per mL of serum.²⁴

Post-challenge clinical signs and body temperatures were recorded 3x weekly for the pigs that were randomly selected for necropsy. Necropsies and all scoring and lesion evaluations were done by Dr.
The following samples were collected and evaluated:

- **Lungs.** The lungs were removed and evaluated grossly for characteristic lesions attributable to *M. hyopneumoniae* and PCV2 infections. The percent lesion involvement per lobe was estimated visually, and the total lung score was based on a calculation of the relative weight of each lung lobe to the total lung weight.

- **Macroscopic lesions.** The lymph nodes were scored macroscopically for hyperplasia as a pool, with 0 indicating normal and 3 indicating three times normal size. The macroscopic scoring system for all other organs was as follows: 0 = normal; 1 = lesion present.

- **Tissue samples.** Microscopic lesions of the liver, heart, lung, tonsils, thymus, spleen, kidney, and lymph nodes were scored as previously described.

- **Bronchoalveolar lavage (BAL) fluids.** BAL fluids were collected for *M. hyopneumoniae* quantitative PCR (RT-PCR) using a procedure developed by Dr. Erin Strait; the results are presented as ng/µl *M. hyopneumoniae* DNA. Bronchial swab samples were also taken to determine the presence of other bacteria. Both the BAL and swab samples were tested at the laboratory of Dr. Thacker.

All pigs were weighed on Study Days -2, 34, 62, 76, 100, and 131 for calculation of average daily gain (ADG). Sort loss at closeout (Study Day 131) was defined as any pig with a weight of less than 230 pounds.

For pigs in the groups scheduled for necropsy and any pig that died or was euthanized at the farm, a case definition of PMWS was made if all three of the following criteria were met:

- Clinical signs of wasting/weight loss/ill thrift/failure to thrive (a required indication), with or without dyspnea or icterus
- Histologic lesions; depletion of lymphocytes from lymphoid tissues (a required indication) and/or lymphohistiocytic to granulomatous inflammation in any organ
- PCV2 infection with characteristic lesions (detected by IHC)

### Market Evaluation

An analysis was conducted of the estimated carcass weights of the pigs remaining on the farm until the Day 131 closeout. Market values in this analysis were calculated according to the grid in Table 2.

### Study Analysis

Analysis of all variables was performed with SAS/STAT User’s Guide Version 8.2 (SAS Institute, Cary, North Carolina). The 5% level of significance ($P \leq 0.05$) was used to assess all statistical differences.

### Results

**M. hyopneumoniae and PCV2 Antibody Titers**

The ELISA antibody titers to *M. hyopneumoniae* are summarized in Figure 1. On Study Days -2 and 13, all pigs were negative for *M. hyopneumoniae* antibodies. For all subsequent time-points, the geometric mean titers (GMT) to *M. hyopneumoniae* of all the bacterin treatment groups were significantly higher than the placebo control group. The GMT of the RespiSure-vaccinated group was significantly higher than the Bacterin A group on Day 48, Day 100, and Day 131 and significantly higher than the Bacterin B group on Days 34, 48, 62, 76, 100, and 131.

The ELISA GMTs to PCV2 are presented in Figure 2. There were no significant differences among any of the treatment groups at any time-point. Maternal antibodies were evident at Day -2, and both

### Table 2—Current industry grid for market weight value

<table>
<thead>
<tr>
<th>Live Weight Range (lb)</th>
<th>Estimated Market Value (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>$42.95/cwt</td>
</tr>
<tr>
<td>201-210</td>
<td>$46.31/cwt</td>
</tr>
<tr>
<td>211-220</td>
<td>$48.49/cwt</td>
</tr>
<tr>
<td>221-230</td>
<td>$52.00/cwt</td>
</tr>
<tr>
<td>231-290</td>
<td>$52.93/cwt</td>
</tr>
<tr>
<td>291-300</td>
<td>$50.71/cwt</td>
</tr>
<tr>
<td>301-310</td>
<td>$49.04/cwt</td>
</tr>
<tr>
<td>311-320</td>
<td>$45.54/cwt</td>
</tr>
<tr>
<td>&gt;320</td>
<td>$42.02/cwt</td>
</tr>
</tbody>
</table>

![Fig. 1—ELISA antibody titers to *M. hyopneumoniae*](image-url)
Day –2 and Day 34 through Day 131 of all three bacterin groups was significantly higher than the placebo group, and there were no differences between the vaccine groups (Table 3).

**Clinical Signs and Body Temperatures**

Clinical signs (sneezing, cough, lethargy, respiratory distress, icterus, and wasting) were recorded for the pigs allocated for necropsy on Day 63 or 77 (Table 4). The prevalence of clinical signs in the RespiSure and Bacterin B groups was significantly lower than in the placebo control group. The days with a clinical sign in the RespiSure group was also significantly lower than the Bacterin A group. The most frequent observation was cough, followed by lethargy and respiratory distress (data not shown).

There were no significant differences between treatment groups at any of the time-points for body temperatures and there were no time-points at which the mean temperatures of any group were ≥104°F (data not shown).

**Percent Lung Lesions**

The percent lung lesion score at necropsy was the pivotal variable analyzed for the pigs euthanized on Day 63 or Day 77. Results summarized in Table 5 show that at Day 63 (2 weeks after PCV2 challenge) the percent lung involvement of all bacterin groups was significantly lower than the placebo control group. For the Day 131 closeout, the ADG for both the titer and number of positive pigs declined prior to challenge with PCV2 on Day 48. Following challenge, antibodies developed, with approximately 50% of the pigs positive by 2 weeks after challenge, approximately 90% positive by 4 weeks after challenge, and nearly 100% positive by Day 100 (data not shown).

**Body Weights and Average Daily Gain**

From the Day –2 through Day 62 time-points, no significant differences were noted between treatment groups. On Day 76, weights of the pigs vaccinated with RespiSure were significantly higher than the placebo control group (Figure 3). The body weights of all bacterin treatment groups were significantly higher than the placebo group on Days 100 and 131, and there were no differences between the bacterin treatments for these two time-points.

Average daily gain (ADG) was one of the pivotal variables analyzed, and ADG was calculated from both pre-vaccination (Day –2) and pre-challenge (Day 34). For the Day 131 closeout, the ADG for both the titer and number of positive pigs declined prior to challenge with PCV2 on Day 48. Following challenge, antibodies developed, with approximately 50% of the pigs positive by 2 weeks after challenge, approximately 90% positive by 4 weeks after challenge, and nearly 100% positive by Day 100 (data not shown).
Bacterin A, and Bacterin B groups (data not shown). On Day 77, all three bacterin groups were significantly lower than the placebo group, and there were no significant differences between the bacterin treatments (data not shown).

**PCV2 DNA Levels in Serum**

Sera from the pigs necropsied on Day 63 or 77 were analyzed by RT-PCR for the presence of PCV2 nucleic acid (Table 6). All pigs were negative at the Day 48 pre-challenge time-point. High levels of PCV2 DNA were detected in the sera of pigs following challenge, but there was considerable variability in the individual pig values, and there were no significant differences between treatments at either time-point.

**Macroscopic and Microscopic Lesions**

There were no differences among treatments for the presence of macroscopic and microscopic lesions in any tissue at either the Day 63 or Day 77 necropsy (data not shown). More than 50% of all animals, regardless of treatment, had positive scores for lymphoid depletion and histiocytic replacement, and were positive by immunohistochemistry for the presence of PCV2.

**Occurrence of PMWS**

The number of pigs meeting the case definition of PMWS is summarized in Table 7. There were no significant differences between the treatments at either the Day 63 or Day 77 necropsy, or among the pigs that remained at the farm. Although many pigs in all treatment groups undergoing necropsy had microscopic lesions associated with PCV2, only a few of the pigs also had clinical observations of wasting or low body weights to meet the case definition of PMWS. For the pigs remaining at the study site, the criteria for meeting the case definition of PMWS could only be evaluated for the pigs that died and were examined on necropsy.

**Sort Loss at Closeout**

There were no significant differences in the number of pigs with body weights

---

### Table 3–Average daily gain

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Daily Gain (lb)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 34 to 131</td>
<td>Day -2 to 131</td>
</tr>
<tr>
<td>T01 Placebo control</td>
<td>1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>T02 RespiSure</td>
<td>2.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
<tr>
<td>T03 Bacterin A</td>
<td>2.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0018</td>
</tr>
<tr>
<td>T04 Bacterin B</td>
<td>2.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

NA = not applicable  
<sup>a</sup><sup>b</sup> Values with different superscripts are significantly (P<0.05) different.  
*P value compared to placebo

### Table 4–Percent of days that an animal had any clinical sign

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Pigs</th>
<th>Least Squares Mean</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T01 Placebo control</td>
<td>28</td>
<td>18.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>T02 RespiSure</td>
<td>26</td>
<td>4.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0005</td>
</tr>
<tr>
<td>T03 Bacterin A</td>
<td>26</td>
<td>12.39&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>0.2228</td>
</tr>
<tr>
<td>T04 Bacterin B</td>
<td>26</td>
<td>7.59&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>0.0168</td>
</tr>
</tbody>
</table>

NA = not applicable  
<sup>a,b,c</sup> Values with different superscripts are significantly (P<0.05) different.  
*P value compared to placebo

### Table 5–Percent lung lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Pigs</th>
<th>Day 63</th>
<th>P Value*</th>
<th>No. Pigs</th>
<th>Day 77</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T01 Placebo control</td>
<td>14</td>
<td>19.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>14</td>
<td>5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>T02 RespiSure†</td>
<td>12</td>
<td>0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
<td>14</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.023</td>
</tr>
<tr>
<td>T03 Bacterin A</td>
<td>12</td>
<td>6.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0008</td>
<td>14</td>
<td>4.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.642</td>
</tr>
<tr>
<td>T04 Bacterin B</td>
<td>12</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
<td>14</td>
<td>2.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.0871</td>
</tr>
<tr>
<td>NTX Sentinel‡</td>
<td>7</td>
<td>0.2</td>
<td>NA</td>
<td>7</td>
<td>0.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

NTX = no treatment  
NA = not applicable  
<sup>a,b,c</sup> Values with different superscripts are significantly (P<0.05) different.  
*P value compared to placebo  
†At Day 63, RespiSure (P=0.0002) and Bacterin B (P=0.0098) scores were lower than Bacterin A.  
‡NTX sentinel group not included in analysis

### Table 6–PCV2 genome copy number per mL of sera from pigs scheduled for necropsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Study Day (Geometric Mean Titer per mL Serum)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 48</td>
</tr>
<tr>
<td>T01 Placebo control</td>
<td>0.00</td>
</tr>
<tr>
<td>T02 RespiSure</td>
<td>0.00</td>
</tr>
<tr>
<td>T03 Bacterin A</td>
<td>0.00</td>
</tr>
<tr>
<td>T04 Bacterin B</td>
<td>0.00</td>
</tr>
<tr>
<td>NTX Sentinel</td>
<td>0.00</td>
</tr>
</tbody>
</table>

NTX = sentinel group not included in analysis  
PCV2 challenge on Day 49  
*There were no significant (P<0.05) differences among treatments for any time-point.
Market Evaluation

The analysis of market values confirmed that the higher mean weights in all bacterin treatment groups translated into an increased market value as estimated by the current industry grid (Figure 4); however, the overall treatment effect was $P = 0.0603$, which was above the significance level tested in this study. Pigs vaccinated with RespiSure had the highest overall market value, with an advantage of $9.63 per pig compared to placebo-vaccinated pigs, $3.80 compared to pigs vaccinated with Bacterin A, and $2.50 compared to pigs vaccinated with Bacterin B.

Discussion

The co-factors or triggers involved in the development of PMWS in PCV2-infected pigs are not completely understood. The clinical expression of PCV2 disease has been reported to be associated with concurrent infections with other pathogens or with non-specific immunostimulation or immunosuppression. Additionally, there are reports that vaccination with oil-based adjuvants, or bacterins formulated with oil-based adjuvants, is associated with a more severe granulomatous inflammatory response in PCV2-infected pigs and with the subsequent development of PMWS. However, it has also been reported that co-inoculation of pigs with PCV2 and a commercial mineral oil-based adjuvant did not potentiate PCV2 replication or the development of PMWS.

This study was conducted to evaluate a possible association between use of *M. hyopneumoniae* bacterins, particularly those formulated with oil-based adjuvants, and the development of PMWS. RespiSure (oil-based) and two competitor’s *M. hyopneumoniae* bacterins, formulated with an oil-based (Bacterin A) or aqueous-based adjuvant (Bacterin B), were evaluated. The model used was the *M. hyopneumoniae*-PCV2 co-infection model developed by Iowa State University researchers. This study was concluded when the pigs reached typical market weight, in order to assess not only the short-term effect that vaccination might have on the development of clinical signs, pneumonia, and other PCV2-associated lesions, but also the effect on production measurements, specifically body weight and ADG.

Co-infection of pigs with *M. hyopneumoniae* and PCV2 resulted in severe respiratory disease in pigs as measured by clinical signs, pneumonia associated with the presence of both *M. hyopneumoniae* and PCV2, and severe microscopic lesions, including lymphoid depletion and histiocytic replacement, associated with PCV2.

There was no evidence of enhanced clinical disease associated with the *M. hyopneumoniae* vaccinations. In contrast, the data indicate that control of *M. hyopneumoniae* through vaccination may reduce the economic effect of respiratory disease in pigs co-infected with *M. hyopneumoniae* and PCV2. Although significant treatment effects were not evident in estimating the market value of study pigs,
closeout data did confirm that the higher mean weights in all bacterin treatment groups were associated with an increased mean market value.

Conclusions

• Based on the case definition of PMWS, there were no significant differences between the placebo and bacterin treatments in the number of pigs developing PMWS or in the number of pigs with low body weights (sort loss) at closeout.

• There was no evidence of enhancement of PCV2 disease associated with M. hyopneumoniae bacterins containing either oil- or aqueous-based adjuvants, as measured by the number of pigs that developed PMWS, the severity of PCV2-associated lesions, or PCV2 levels in serum.

• All groups vaccinated with M. hyopneumoniae bacterins had higher body weights and ADG at Day 100 and the Day 131 closeout time-point.

• Compared to the placebo control group, all groups vaccinated with M. hyopneumoniae bacterins also had significantly lower gross lung lesion scores on Day 63 and significantly lower levels of M. hyopneumoniae detected in BAL samples on Day 77. Pigs vaccinated with RespiSure and Bacterin B also had significantly fewer days with a clinical sign and lower DNA levels in BAL on Day 63 compared to placebo control pigs.

• Significant differences between RespiSure and the two other M. hyopneumoniae bacterins were evident in the following measured variables:
  - Lung lesion scores for the RespiSure group were lower than Bacterin A on Day 63 (P = 0.0002), and only RespiSure scores were lower than the placebo group on Day 77.
  - At the Day 63 necropsy, the level of M. hyopneumoniae DNA in the BAL samples for the RespiSure group was lower than the levels for both Bacterin A (P = 0.0033) and Bacterin B (P = 0.0087).

- The RespiSure group had a lower percentage of days with a clinical sign than the Bacterin A group (P = 0.0224).
- M. hyopneumoniae ELISA antibody titers in the pigs vaccinated with RespiSure were higher than Bacterin A at Day 48 (P = 0.0001), Day 100 (P = 0.0303), and Day 131 (P = 0.0091). Similarly, the M. hyopneumoniae antibody titers for RespiSure were higher than Bacterin B for all blood samples collected from Day 34 through Day 131 (P = 0.0001 at each time-point).

• These data from pigs co-infected with M. hyopneumoniae and PCV2 in a production setting do not support previous reports that vaccination with M. hyopneumoniae bacterins enhances losses associated with PMWS. Rather, all bacterin groups out-performed the placebo control group in ADG.

• Therefore, the use and appropriate timing of M. hyopneumoniae vaccination are effective in reducing the production losses associated with the respiratory disease complex induced by co-infection with M. hyopneumoniae and PCV2.

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28. Ghebremariam MK, Gruys E. Postweaning multisystemic wasting syndrome (PMWS) in pigs with particular emphasis on the causative agent, the mode of transmission, the diagnostic tools, and the control measures. *Vet Quarterly* 2005;27:105-116.


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This study was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Veterinary Resources, Inc.