Comparative efficacy of concurrent administration of a porcine circovirus type 2 (PCV2) vaccine plus a porcine reproductive and respiratory syndrome virus (PRRSV) vaccine from two commercial sources in pigs challenged with both viruses

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Summary

Objective: To compare clinical, virologic, immunologic, and pathologic parameters in pigs each concurrently administered a porcine circovirus type 2 (PCV2) and a porcine reproductive and respiratory syndrome virus (PRRSV) vaccine from one of two commercial sources and challenged with field strains of both viruses.

Materials and methods: One group of pigs administered concurrently Fostera PCV and Fostera PRRS (Zoetis, Florham Park, New Jersey) and another group administered concurrently Ingelvac CircoFLEX and Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) at study day -28 (21 days of age) were challenged with both viruses at study day 0 (49 days of age). Serum samples were tested for viremia by real-time polymerase chain reaction (PCR), and for antibodies by a commercial enzyme-linked immunosorbent assay and a virus neutralization test. Peripheral blood mononuclear cells were tested for interferon-γ secreting cells (IFN-γ-SC) by enzyme-linked immunospot assay. Lung and lymphoid tissues were tested for lesions and viral antigen by histopathology and immunohistochemistry.

Results: Significant differences were observed between vaccinated, challenged and unvaccinated, challenged groups in clinical (average weight gain and clinical signs), virologic (except PCV2 viremia at day 14), immunologic, and pathologic outcomes.

Implications: Under the conditions of this study, it makes no difference to protection whether PCV2 and PRRSV vaccines are administered concurrently. Concurrent vaccination is efficacious for controlling co-infection with PCV2 and PRRSV.

Keywords: swine, porcine circovirus-associated diseases, porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, vaccine

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Resumen - Eficacia comparativa de la administración simultánea de una vacuna de circovirus porcino tipo 2 (PCV2) más una vacuna del virus del síndrome reproductivo y respiratorio porcino (PRRSV) de dos casas comerciales en cerdos retados con ambos virus

Objetivo: Comparar los parámetros clínicos, virológicos, inmunológicos y patológicos en cerdos a los que se les administró simultáneamente una vacuna de circovirus porcino tipo 2 (PCV2 por sus siglas en inglés) y del virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés) de una de las dos casas comerciales y fueron retados con cepas de campo de ambos virus.

Materiales y métodos: Los dos grupos de cerdos, uno al que se le administró simultáneamente Fostera PCV y Fostera PRRS (Zoetis, Florham Park, New Jersey) y otro, al que se le administró Ingelvac CircoFLEX e Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) en el día -28 del estudio (21 días de edad) fueron retados con ambos virus en el día 0 del estudio (49 días de edad). Las muestras de suero se analizaron para medir viremia por medio de la reacción en cadena de polimerasa en tiempo real (PCR), y en busca de anticuerpos por medio de ensayo por inmunosorción ligado a enzimas comercial y una prueba de neutralización de virus. Se buscaron las células mononucleares de sangre periférica en busca de células que secretan interferón γ (IFN-γ-SC, por sus siglas en inglés) por medio del ensayo inmunospot ligado a enzimas. Se buscaron los tejidos linfoides y de pulmón en busca de antígenos virales, y lesiones por medio de histopatología e inmunohistoquímica.

Resultados: Se observaron diferencias significativas entre los grupos retados sin vacunar y los retados vacunados en evaluación clínica (ganancia de peso promedio y signos
Porcine respiratory disease complex (PRDC) is a serious health problem in growing and finishing pigs, typically approximately 16 to 22 weeks of age, and is characterized by slow growth, poor feed efficiency, lethargy, anorexia, fever, cough, and dyspnea. Pathogens involved in PRDC can be viral, bacterial, or both. Among them, a co-infection with porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) is the most common etiology of PRDC. Therefore, controlling both PCV2 and PRRSV infection is a high priority for the swine industry globally. Since vaccination is one of the major tools to control PCV2 and PRRSV infection, vaccination of pigs with both PCV2 and PRRSV is necessary to control PRDC efficiently.

Recently, a new commercial modified-live PRRSV vaccine (Fostera PRRS; Zoetis, St Joseph, Missouri) at the year -28 of the study (21 days of age) and the animals infected with the two viruses at day 0 of the study (49 days of age). Des échantillons de sérum ont été testés par réaction d’amplification en chaîne par la polymérase (ACP) pour détecter une viremie, et par une épreuve immuno- enzymatique commerciale ainsi qu’un test de neutralisation virale pour détecter des anti-corps. Les cellules mononucléaires du sang périphérique ont été testées pour la présence de cellules productrices d’interféron-γ (IFN-γ) au moyen d’une épreuve immuno- enzymatique par tache. Les tissus lymphoïde et pulmonaire ont été examinés pour la présence de lésions et d’antigène viral par histopathologie et immunohistochimie.

Résultats: Des différences significatives ont été observées entre les groupes d’animaux vaccinés et infectés et les animaux non-vaccinés et infectés du point de vue clinique (gain moyen quotidien et signes cliniques), virologique (épreuve ACP), immunologique (anticorps, IFN-γ-SC, et interleukine-10), et pathologique (lésions et antigène viral). Aucune différence significative ne fut notée entre les deux groupes d’animaux vaccinés et infectés pour ce qui est des aspects clinique, virologique (sauf la viremie CVP2 au jour 14), immunologique et pathologique.

Implications: Dans les conditions expérimentales de la présente étude, aucune différence dans la protection ne fut causée par l’administration simultanée des vaccins PCV2 et VSRRP. La vaccination simultanée est efficace pour limiter la co-infection par PCV2 et VSRRP.

Materials and methods
All animal protocols were approved by the Seoul National University Institutional Animal Care and Use Committee.

Experimental study
Sixty colostrum-fed, crossbred, conventional piglets were purchased at 5 days of age from a commercial Korean farm. Upon arrival at a research facility, all piglets in this study tested negative for PCV2 and PRRSV by serological testing (PCV2 Ab Mono Blocking ELISA; Synbiotics, Lyon, France, and PRRS X3 Ab Test; Idexx Laboratories Inc, Westbrook, Maine). All piglets also tested negative for PCV2 and PRRSV viremia by real-time polymerase chain reaction (PCR). A total of 60 pigs were randomly divided into four groups using the random number generation function in Excel (Microsoft Corporation, Redmond, Washington) (Table 1). Sample size was calculated assuming a 90% power (1 - β = .90) of detecting a difference at the 5% level of significance (α = .05), which was based on expected results of ELISA antibody titers (PCV2 and PRRSV), virus load (PCV2 and PRRSV) determined by real-time PCR, and lung and lymphoid lesions represented by scores. The treatment timeline is shown in Table 1. Pigs in Group 1 were administered one 2.0 mL dose of Fostera PCV (Zoetis) and one 2.0 mL dose of Fostera PRRS (Zoetis) intramuscularly in the right and left sides of the neck, respectively, at study day -28 (21 days of age) according to the manufacturer’s label instructions. Pigs in Group 2 were administered one 1.0 mL dose of Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) and one 2.0 mL dose of Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica Inc) intramuscularly in the right and left sides of the neck, respectively, at study day -28 according to the manufacturer’s label instructions. At study day 0 (49 days of age), each pig in groups 1, 2, and 3 was inoculated intranasally with 2 mL of PCV2b (strain SNUVR000463; 5th passage; 1.2 × 10^5 TCID50 per mL). In the afternoon of the same day, the same pigs were inoculated intranasally with 2 mL of PRRSV (strain SNUVR090851; 5th passage; 1.2 × 10^5 TCID50 per mL). Co-infection with these PCV2b and PRRSV strains induced severe interstitial pneumonia and lymphoid depletion of lymph nodes in infected pigs. Group 3 pigs served as the positive-control.
group (challenged but not vaccinated), and Group 4 pigs served as the negative-control group (unchallenged and unvaccinated). Groups were housed in separate rooms (five pigs per room) within the same facility. Blood samples were collected at study days -42, -28, 0 (49 days of age), 14, 28, 63, 91, and 126 (175 days of age). Nasal swabs were also collected on these study days.

Assessment of growth performance
Body weight of each pig in groups 1, 2, 3, and 4 was measured at study days -28, 0, 21, 63, and 126. Average daily gain (ADG; grams per pig) was analyzed over four time periods: between day -28 and 0; 0 and 21; 21 and 63; and 63 and 126, respectively. The ADG during these various production stages was calculated as the difference between the starting and final weights divided by the duration of the stage. Data from dead pigs were included in the calculation.

PRRSV serological testing
Serum samples were tested using a commercial PRRSV ELISA (Idexx Laboratories Inc) and serum virus neutralization using the heterologous challenging PRRSV (strain SNUVR090851). Serum samples were considered positive for anti-PRRSV antibody if the sample-to-positive (S:P) ratio was > 0.4, according to the manufacturer’s instructions. The NAb data were converted to base 2 logarithms for analysis.

Quantification of PCV2 DNA
QIAamp DNA Mini Kit (Qiagen Inc, Valencia, California) was used to extract DNA from serum samples. The DNA extracts were used to quantify numbers of PCV2 genomic DNA copies by real-time PCR as previously described. The numbers of genomic copies of PCV2 DNA per mL of serum were converted to base 10 logarithms for analysis.

Quantification of PRRSV RNA
A QIAamp RNA Mini Kit (Qiagen Inc) was used to extract RNA from serum samples. The RNA extracts were used to quantify numbers of PRRSV RNA copies by real-time qPCR as previously described. The numbers of RNA copies of PRRSV RNA per mL of serum were converted to base 10 logarithms for analysis.

Table 1: Means (with standard deviations) of lymphoid and pulmonary lesion scores and numbers of cells positive for lymphoid porcine circovirus type 2 (PCV2) antigen and pulmonary porcine reproductive and respiratory syndrome virus (PRRSV) antigen in pigs vaccinated concurrently with PCV2 and PRRSV vaccines and challenged with PCV2 and PRRSV

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination (21 days of age)</th>
<th>Challenge (49 days of age)</th>
<th>Lymph node</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lesion score†</td>
<td>No. of PCV2† cells‡</td>
</tr>
<tr>
<td>1</td>
<td>Fostera PCV and Fostera PRRSV</td>
<td>PCV2 and PRRSV</td>
<td>0.43 (0.53)a</td>
<td>3.15 (3.52)a</td>
</tr>
<tr>
<td>2</td>
<td>Ingelvac CircoFLEX and Ingelvac PRRS MLV</td>
<td>PCV2 and PRRSV</td>
<td>0.71 (0.59)a</td>
<td>7.05 (5.45)a</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>PCV2 and PRRSV</td>
<td>2.11 (0.73)b</td>
<td>20.70 (8.17)b</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>None</td>
<td>0.28 (0.41)a</td>
<td>0</td>
</tr>
</tbody>
</table>

* Group 1 pigs were concurrently administered Fostera PCV and Fostera PRRS vaccines (Zoetis, Florham Park, New Jersey) and Group 2 pigs were concurrently administered Ingelvac CircoFlex and Ingelvac PRRS MLV vaccines (Boehringer Ingelheim Vetmedica Inc, St. Joseph, Missouri), and both groups were challenged with both viruses. The body weight of each pig was measured at study days -28 (21 days of age), 0, 21 (70 days of age), 63, and 126 (175 days of age). Blood samples with EDTA were collected from pigs for interferon-γ secreting cells and without anticoagulant for serologic testing at study days -42, -28, 0, 14, 28, 63, 91, and 126. Nasal swabs were also collected on these study days.
† Pigs in all groups were euthanized at 175 days of age. Superficial inguinal lymph node and lung were collected for histopathologic examination and immunohistochemical testing. Lymphoid lesion scores: 0 = no lymphoid depletion or granulomatous replacement; 1 = mild lymphoid depletion; 2 = moderate lymphoid depletion; and 3 = severe lymphoid depletion and histiocytic replacement. Lung lesion scores: 0 = no microscopic lesions; 1 = mild interstitial pneumonia; 2 = moderate multifocal interstitial pneumonia; 3 = moderate diffuse interstitial pneumonia; and 4 = severe interstitial pneumonia. Scores were compared among groups using Fisher’s exact test.
‡ Numbers of lymphoid and pulmonary cells positive for PCV2 antigen, and of pulmonary cells positive for PRRSV antigen, per unit area (0.25 mm²) of lung were counted using an NIH Image J 1.45s program (http://imagej.nih.gov/ij/download.html). Numbers of positive cells were compared among groups using Tukey’s test.

ABC Within a column, values with different superscript letters are significantly different (P < .05).
used to extract RNA from serum samples. The RNA extracts were used to quantify numbers of PRRSV genomic RNA copies by real-time PCR as previously described. The numbers of genomic copies of PRRSV RNA per mL of serum were converted to base 10 logarithms for analysis.

Enzyme-linked immunospot assay

The numbers of PCV2- and PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) were determined in peripheral blood mononuclear cells (PBMC) by the enzyme-linked immunospot (ELISPOT) method as previously described. Whole PCV2b and PRRSV (the strains used for challenge), each at a multiplicity of infection of 0.01, were used to stimulate PBMC. Phytohemagglutinin (10 µg per mL; Roche Diagnostics GmbH, Mannheim, Germany) and phosphate buffered saline were used as positive and negative controls, respectively. The results were expressed as the numbers of IFN-γ-SC per million PBMC.

Interleukin-10

The protein concentrations of interleukin-10 (IL-10) were quantified in the supernatants of PBMC cultures (2 × 10^6 cells per well; 250 µL) in vitro for 20 hours with the challenging PRRSV (multiplicity of infection of 0.01) or phytohemagglutinin (10 µg per mL) using commercial ELISA kits (Pig Interleukin-10 ELISA kit; Cusabio Biotech, Wuhan, China) according to the manufacturer's instructions. The detection limit for IL-10 was 1.5 pg per mL.

Histopathologic examination

For morphometric analysis of histopathologic lesion scores in lymph nodes, the superficial inguinal lymph node was collected from each pig, and three sections of that lymph node were examined blindly as previously described. Lymphoid lesions were scored on a scale from 0 to 3: 0 = no lymphoid depletion or replacement; 1 = mild lymphoid depletion; 2 = moderate lymphoid depletion; and 3 = severe lymphoid depletion and histiocytic replacement.

For morphometric analysis of histopathologic lesion scores in lung, eight samples of lung tissue (two from the right cranial lobe, two from the right middle lobe, one from the ventromedial part of the right caudal lobe, one from the dorsomedial part of the right caudal lobe, and one from the accessory lobe) were collected from each pig and three sections of that lung tissue were examined histologically by one of the authors (JJ), blinded to the animal IDs, as previously described. Lung lesions were scored on a scale from 0 to 4: 0 = no microscopic lesions; 1 = mild interstitial pneumonia; 2 = moderate multifocal interstitial pneumonia; 3 = moderate diffuse interstitial pneumonia; and 4 = severe interstitial pneumonia.

Immunohistochemical examination for PCV2 antigen was performed using PCV2 polyclonal antibody (Iowa State University, Ames, Iowa), and PCV2 antigen was performed using SR30 monoclonal antibody (Rural Technologies Inc, Brookings, South Dakota). Numbers of lymphoid cells positive for PCV2 antigen in lymph node and cells positive for PRRSV and PCV2 antigen in lung were counted using an NIH Image J 1.45s program (http://imagej.nih.gov/ij/download.html).

Statistical analysis

Continuous data (rectal body temperature, body weight; PCV2 DNA (log_{10} PCV2 genomic copies per mL) determined by real-time PCR; PRRSV RNA (log_{10} PRRSV genomic copies per mL) determined by real-time PCR; PCV2 and PRRSV serum titer; number of IFN-γ-SC per 10^6 PBMC determined by ELISPOT assay; numbers of lung sections positive for PRRSV antigen and PCV2 antigen; and lymph-node sections positive for PCV2 antigen per unit area (0.25 mm²; determined by immunohistochemistry) were analyzed using repeated measures ANOVA for each time point. If the ANOVA showed a significant effect, Tukey's test for multiple comparisons was performed at each time point. Fisher's exact test was used for discrete data (clinical respiratory score and lung and lymphoid lesion scores). A chi-square test was used for mortality rate. A value of P < .05 was considered significant.

Results

Clinical evaluation

Mean respiratory scores were significantly higher (P < .05) in unvaccinated, challenged pigs (Group 3) than in vaccinated, challenged pigs (Group 1 and Group 2) from day 7 to 42 and from day 84 to 98 (Figure 1A).
Immunological responses to PCV2
On days 0 through 28, anti-PCV2 antibody titers were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). Anti-PCV2 antibody titers differed between the two groups of vaccinated, challenged pigs (Group 1 and Group 2) at day 0 (Figure 3A). On days 0 through 91, mean NAb titers were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). Mean NAb titers differed between the two groups of vaccinated, challenged pigs (Group 1 and Group 2) at day 14 (Figure 3B). On days 0 through 28, numbers of PCV2-specific IFN-γ-SC were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). Numbers of PCV2-specific IFN-γ-SC differed between the two groups of vaccinated, challenged pigs (Group 1 and Group 2) at days 0 and 14 (Figure 3C). No anti-PCV2 antibodies or PCV2-specific NAb or IFN-γ-SC were detected in Group 4 (unvaccinated, unchallenged pigs).

Immunologic responses to PRRSV
On days 0 through 63, anti-PRRSV antibody titers were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). (Figure 4A). On days 91 and 126, mean NAb titers were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs) (Figure 4A). On days 91 and 126, mean NAb titers were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). (Figure 4A).
higher \((P < .05)\) in Group 1 and Group 2 (unvaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs) (Figure 4B). On days 0 through 28, numbers of PRRSV-specific IFN-\(\gamma\)-SC were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs) (Figure 4C). No anti-PRRSV antibodies or PRRSV-specific NAb or IFN-\(\gamma\)-SC were detected in Group 4 (unvaccinated, unchallenged pigs).

### PRRSV-specific IL-10

On day 0, IL-10 levels were significantly higher \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). Concentrations of IL-10 differed between the two groups of vaccinated, challenged pigs (Group 1 and Group 2) at day 0. On day 28, IL-10 concentrations were significantly higher \((P < .05)\) in Group 3 (unvaccinated, challenged pigs) than in Group 1 and Group 2 (vaccinated, challenged pigs) (Figure 5). No IL-10 was detected in Group 4 (unchallenged, unvaccinated pigs).

### Pathologic testing

Lymphoid and pulmonary lesion scores were significantly lower \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs). The numbers of lymphoid cells positive for PCV2 antigen (Figure 6), and pulmonary cells positive for PRRSV antigen (Figure 7) and PCV2 antigen (Figure 8) were significantly lower \((P < .05)\) in Group 1 and Group 2 (vaccinated, challenged pigs) than in Group 3 (unvaccinated, challenged pigs) (Table 1).

### Discussion

This study demonstrated that the single-dose vaccination regimen for PCV2 and PRRSV vaccine is efficacious for controlling co-infection with PCV2 and PRRSV. Regardless of types of vaccines, ADG was higher and mortality rate was lower in the vaccinated, challenged animals than in the unvaccinated, challenged animals.

Porcine circovirus type 2 viremia is correlated with the severity of PCV2-induced lymphoid lesions.\(^{16,17}\) Therefore, PCV2 viremia is an appropriate parameter to evaluate a PCV2 vaccine. A lower number of genomic copies of PCV2 DNA correlates with induction of PCV2-specific NAb and IFN-\(\gamma\)-SC.\(^{16-20}\) In the current study, only vaccinated animals exhibited PCV2-specific NAb and IFN-\(\gamma\)-SC. Pigs immunized with the Fostera PCV and Fostera PRRSV vaccine (Group 1) had higher titers of PCV2-specific NAb and higher numbers of IFN-\(\gamma\)-SC than did pigs immunized with the Ingelvac CircoFLEX and Ingelvac PRRS MLV vaccines (Group 2). These differences likely influenced the lower numbers of genomic copies of PCV2 DNA in Group 2. These results agree with previous findings that the Fostera PCV vaccine results in significantly lower numbers of genomic copies of PCV2 DNA and greater protective immunity (higher titers of PCV2-specific NAb and higher numbers of IFN-\(\gamma\)-SC) when compared to the Ingelvac CircoFLEX vaccine.\(^{21}\)

The number of genomic PRRSV RNA copies in serum samples is a critical parameter to evaluate the efficiency of vaccines in control of PRRSV infection.\(^{22}\) In the present study, PRRSV viremia had resolved before neutralizing antibodies were developed. Therefore, neutralizing antibodies are not essential for the lower number of genomic PRRSV RNA copies as reported in previous studies.\(^{23,24}\) In addition, there is no evidence that PRRSV antibodies detected by ELISA play a role in protection against infection with PRRSV.\(^{25}\)

In contrast, a lower number of PRRSV genomic RNA copies coincided with the appearance of PRRSV-specific IFN-\(\gamma\)-SC in vaccinated, challenged animals. Therefore, PRRSV-specific IFN-\(\gamma\)-SC are responsible for PRRSV clearance, although the role of IFN-\(\gamma\)-SC in a lower number of PRRSV RNA copies is still conflicting.\(^{23,26}\)

In the present study, no significant differences were observed in the ability of the two tested PRRSV vaccines to induce PRRSV-specific IFN-\(\gamma\)-SC and reduce PRRSV viremia, as a previous study showed.\(^{10}\)

Pathologic evaluation is another critical parameter to determine the efficacy of the PCV2 and PRRSV vaccines under experimental conditions. The characteristic microscopic lesions caused by co-infection with PCV2 and PRRSV were severe interstitial pneumonia and lymphoid depletion in the unvaccinated, challenged animals in the present and previous studies.\(^{2,27}\) Single-dose vaccination with PCV2 and PRRSV at 21 days of age was effective in lowering scores for lung and lymphoid lesions in the vaccinated, challenged animals, compared to the unvaccinated, challenged animals, without significant differences between Fostera PCV-PRRS and Ingelvac CircoFLEX-PRRS MLV.

There is interest in the possible interference with the efficacy of one vaccine by another, because animals received both PCV2 and PRRSV vaccines at the same time in this study. Especially, induction of IL-10 by PRRSV vaccine raised concerns that vaccination with PRRSV may interfere with the

### Table 2: Means (with standard deviation) of average daily gain (ADG) in pigs in the study described in Table 1

<table>
<thead>
<tr>
<th>Period between study days*</th>
<th>Age (days)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>-28 to 0</td>
<td>21-49</td>
<td>329 (31)</td>
<td>330 (28)</td>
<td>326 (25)</td>
<td>340 (27)</td>
</tr>
<tr>
<td>0 to 21</td>
<td>49-70</td>
<td>629 (33)</td>
<td>612 (35)</td>
<td>519 (24)</td>
<td>626 (43)</td>
</tr>
<tr>
<td>21 to 63</td>
<td>70-112</td>
<td>792 (44)</td>
<td>785 (47)</td>
<td>672 (45)</td>
<td>804 (39)</td>
</tr>
<tr>
<td>63 to 126</td>
<td>112-175</td>
<td>734 (43)</td>
<td>718 (39)</td>
<td>650 (33)</td>
<td>728 (42)</td>
</tr>
<tr>
<td>-28 to 126</td>
<td>21-175</td>
<td>662 (33)</td>
<td>651 (34)</td>
<td>579 (39)</td>
<td>664 (43)</td>
</tr>
</tbody>
</table>

* The body weight of each pig in each group was measured at study days -28 (21 days of age), 0, 21 (70 days of age), 63, and 126 (175 days of age) and ADGs were compared among groups using Tukey’s test.

ab Within a row, values with different superscript letters are significantly different \((P < .05)\).
Implications

- Under the conditions of this study, it makes no difference to protection whether single-dose PCV2 and PRRSV vaccines are administered concurrently.
- Under the conditions of this study, concurrent vaccination of pigs with PCV2 and PRRSV is efficacious for controlling co-infection with PCV2 and PRRSV.

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Conflict of interest

None reported.

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References

Figure 3: Mean (with standard deviation) for anti-PCV2 reciprocal ELISA antibody titers (Panel A); group means transformed to base 2 logarithms (with standard deviation) for neutralizing antibody (NAb) reciprocal titers (Panel B); and mean (with standard deviation) of PCV2-specific interferon-γ secreting cells (IFN-γ-SC) in peripheral blood mononuclear cells (PBMC) (Panel C) in the study described in Table 1. Different letters (a,b) indicate significant differences among groups (P < .05; repeated measures ANOVA).
Figure 4: Means (with standard deviation) of commercial PRRSV ELISA sample-to-positive (S:P) ratio (Panel A); group means transformed to base 2 logarithms (with standard deviation) for neutralizing antibody (NAb) reciprocal titers (Panel B); and mean (with standard deviation) of PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) in peripheral blood mononuclear cells (PBMC) (Panel C) in the study described in Table 1. Different letters (a,b) indicate significant differences among groups (P < .05; repeated measures ANOVA).
Figure 5: Mean (with standard deviation) for PRRSV-specific IL-10 concentrations in serum samples from pigs in the study described in Table 1. Different letters (a,b) indicate significant differences among groups (P < .05; repeated measures ANOVA).

-42 0 28 61 91 126

IL-10 (pg/mL)

Study day

0 40 80 120 160 200

Group 1Group 2Group 3


Figure 6: Immunohistochemical testing to detect porcine circovirus type 2 (PCV2) antigen in lymph nodes of pigs in the study described in Table 1 was performed using PCV2 polyclonal antibody (Iowa State University, Ames, Iowa). Few PCV2 antigen-positive cells (arrowheads) were detected in macrophages in Group 1 pigs (Panel A). Numerous PCV2 antigen-positive cells were detected in macrophages in Group 3 pigs (Panel B) (magnification × 400).

Figure 7: Immunohistochemical testing to detect porcine reproductive and respiratory syndrome virus (PRRSV) antigen in lungs of pigs in the study described in Table 1 was performed using SR30 monoclonal antibody (Rural Technologies Inc, Brookings, South Dakota). Few PRRSV antigen-positive cells (arrowheads) were detected in macrophages in pigs from Group 1 (Panel A). Numerous PRRSV antigen-positive cells were detected in macrophages in pigs from Group 3 (Panel B) (magnification × 200).

Figure 8: Immunohistochemical testing to detect porcine circovirus type 2 (PCV2) antigen in lungs of pigs in the study described in Table 1 was performed using PCV2 polyclonal antibody (Iowa State University, Ames, Iowa). Few PCV2 antigen-positive cells (arrowheads) were detected in macrophages in pigs from Group 1 (Panel A). Numerous PCV2 antigen-positive cells were detected in macrophages in pigs from Group 3 (Panel B) (magnification × 200).