

Title: Comparison of PCV2 vaccine efficacy in 5 and 21 day old piglets – NPB #09-173

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Industry Summary

Porcine circovirus type 2 (PCV2) vaccines have become widely used since approved in 2006. It is not uncommon for producers to use PCV2 vaccines in pigs younger than what is approved or recommended by manufacturers. The objective of this study was to determine the efficacy of a chimeric and a subunit PCV2 vaccine administered at 5 or 21 (d5 or d21) days of age. In order to mimic the field situation, the pigs were concurrently challenged at 4-6 weeks following vaccination with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV). Forty-eight PCV2 naïve piglets were randomly divided into six groups of eight pigs each. Vaccination was done at d5 or d21 followed by triple challenge at d49. Vaccinated pigs seroconverted to PCV2 approximately 14 days post vaccination. The d5 vaccinated pigs had higher anti-PCV2 antibody levels until d35. At d49, the pigs vaccinated with chimeric vaccine had significantly higher levels of neutralizing antibodies compared with the pigs vaccinated with the subunit vaccine. After challenge the vaccinated pigs, regardless of vaccine type or timing, had significantly decreased levels of PCV2 viremia and significantly decreased prevalence and severity of microscopic lesions compared with pigs in the unvaccinated positive control group. Severe microscopic lesions in lymphoid tissues associated with abundant PCV2 antigen compatible with PCVAD were only present in positive control pigs. The results of this study indicate that under the conditions of this study, off-label vaccination of PCV2 naïve pigs at d5 resulted in earlier development of anti-PCV2 antibodies and provided significant reduction or complete protection against PCV2 viremia and PCV2-associated lesions after triple challenge with PCV2, PPV and PRRSV.

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Scientific Abstract

Porcine circovirus type 2 (PCV2) vaccines have become widely used since approved in 2006. It is not uncommon for producers to use PCV2 vaccines in pigs younger than what is approved or recommended by manufacturers. The objective of this study was to determine the efficacy of a chimeric and a subunit PCV2 vaccine administered at 5 or 21 (d5 or d21) days of age. In order to mimic the field situation, the pigs were concurrently challenged at 4-6 weeks following vaccination with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV). Forty-eight PCV2 naïve piglets were randomly divided into six groups of eight pigs each. Vaccination was done at d5 or d21 followed by triple challenge at d49. Vaccinated pigs seroconverted to PCV2 approximately 14 days post vaccination. The d5 vaccinated pigs had higher anti-PCV2 antibody levels until d35. At d49, the pigs vaccinated with chimeric vaccine had significantly higher levels of neutralizing antibodies compared with the pigs vaccinated with the subunit vaccine. After challenge the vaccinated pigs, regardless of vaccine type or timing, had significantly decreased levels of PCV2 viremia and significantly decreased prevalence and severity of microscopic lesions compared with pigs in the unvaccinated positive control group. Severe microscopic lesions in lymphoid tissues associated with abundant PCV2 antigen compatible with PCVAD were only present in positive control pigs. The results of this study indicate that under the conditions of this study, off-label vaccination of PCV2 naïve pigs at d5 resulted in earlier development of anti-PCV2 antibodies and provided significant reduction or complete protection against PCV2 viremia and PCV2-associated lesions after triple challenge with PCV2, PPV and PRRSV.

Introduction

Porcine circovirus (PCV) is a circular, single stranded, non-enveloped DNA virus (40) that can be separated into two main genotypes: PCV type 1 (PCV1) and PCV type 2 (PCV2). PCV1 is not associated with disease or lesions in pigs and is commonly considered non-pathogenic (41). PCV2 is linked with a variety of clinical disease manifestations collectively referred to as PCV-associated diseases (PCVAD) including systemic disease or post-weaning multisystemic wasting syndrome (PMWS) (14), respiratory disease (15), and enteric disease (18) in growing pigs.

PCV2 is prevalent worldwide, and most herds are seropositive (4, 5, 41). From 2005 to 2006, PCVAD became increasingly problematic in North America leading to high production losses for producers (17). Aggressive vaccination programs initiated in 2006 have substantially decreased the prevalence and severity of PCVAD (11, 19).

Swine producers now have several choices of approved commercial vaccines. Two commonly used commercial PCV2 vaccines include a subunit vaccine and a chimeric vaccine. One of the subunit vaccines (Ingelvac® CircoFLEX™; Boehringer Ingelheim Vetmedica) is licensed for use in pigs 21 days of age (d21) or older and provides protection beginning two weeks post-vaccination, and according to the manufacturer, lasts for a duration of four months. The inactivated chimeric PCV2 vaccine (Suvaxyn® PCV2; Fort Dodge Animal Health, Inc.) is also licensed for use in pigs that are 21 days of age or older, and is also licensed to be administered in one dose (2 ml) at d21 or in two doses (1 ml each) at d21 and d42. According to the manufacturer, this product provides protection beginning three weeks post-vaccination for up to four months duration, and was voluntarily removed from the market in May 2010 due to concerns regarding the inactivation process (10).

In the field, co-infections heavily influence the severity and outcome of PCVAD. Some of the most severe field case reports of PCVAD describe co-infection of pigs with PCV2, PPV, and PRRSV (6, 7). PPV is a linear, single stranded, 5-kb, negatively charged, non-enveloped DNA virus (23). Replication is most active during the S (synthesis) phase of the cell cycle (31). PPV has been shown to cause stillbirths and mummification in breeding herds, but is generally considered non-pathogenic in growing pigs (20); however, when pigs are co-infected with PCV2 and PPV it can lead to severe PCVAD in a portion of the pigs (7).

PRRSV is a single stranded, 15-kb, enveloped RNA virus (3). PRRSV has become endemic and is known to cause abortions in the breeding herd and pneumonia in growing pigs (35). When found combined with

PCV2 in the field or when pigs are experimentally co-infected with PRRSV and PCV2 disease and lesions are often quite severe. (2, 16, 36).

A common concern when encountering a vaccination program, besides the efficacy of the potential vaccine, is the appropriate timing of vaccination to guarantee maximal protection. Vaccines are commonly delivered and labeled for vaccination at d21, but some debate over risks associated with early vaccination exists. Pig farm managers in general prefer to vaccinate pigs at d2 to d5, which is when they are handling piglets for other reason such as castration, iron administration, tail docking, and teeth clipping. There are concerns, ongoing discussions, and debate over whether the pig has a sufficiently mature immune system at this age. Therefore, if vaccinations against pathogens such as PCV2 are proven to be effective in pig less than one week of age, this ultimately could lead to substantial changes in vaccination protocols on many farms.

Objectives

To compare the efficacy of commercial PCV2 one-dose products when administered at processing (5 days of age) or as recommended by the manufacturer at 21 days of age in the PCV2-PRRSV-PPV challenge model.

Materials and Methods

Animals and housing. Forty-eight conventional crossbred pigs were derived from six sows from a breeding herd known to be free of PCV2, PRRSV and PPV as determined by routine serology conducted monthly. At 4 days of age, while still on the dam, all pigs were ear tagged and randomly assigned to one of six treatment groups within each litter so that at least one pig from each sow was in a given treatment group. The pigs were weaned at approximately 14 days of age and transported to the research facility. Upon arrival at the Iowa State University Livestock Infectious Disease Facility, the pigs were separated into four rooms: one room for the negative control group, one room for the positive control group, one room for both groups receiving the chimeric vaccine, and one room for both groups receiving the subunit vaccine. Pigs were housed in pens on a concrete floor that was cleaned once daily in rooms with separate ventilation systems and one nipple drinker. The vaccinated pigs were separated in two pens placed on opposite sides of the room based on timing of vaccination at d5 or d21. All pigs were fed an age appropriate diet free of animal proteins (excluding whey) and antibiotics (Natures Made, Heartland Co-op, Cambridge, IA).

Experimental design. This study was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC # 11-09-6831-S) and the Institutional Biosafety Committee (IBC# 09-I-0030-A). The 48 pigs were randomly divided into groups of 8 pigs. At d5, 16 pigs were vaccinated with one of two PCV2 vaccines: a chimeric vaccine (*Chimeric-d5*) or a viral subunit vaccine (*Subunit-d5*). Similarly at d21, 16 pigs were vaccinated with the chimeric vaccine (*Chimeric-d21*) or a subunit vaccine (*Subunit-d21*). Upon arrival to the research facility, blood was collected at weekly intervals until termination of the project at week 10. The blood samples were collected in serum separator tubes (Benton Dickson Vacutainer™, 8.5 ml), centrifuged at 2000 × g for 10 min at 4°C, and the serum was separated into two aliquots stored at -80°C until testing. All pigs, except for the negative control group, were inoculated with PPV, PRRSV, and PCV2b at d49, and all pigs were euthanized for necropsy at d70.

Clinical evaluation. Upon arrival at the research facility the pigs were individually examined and then monitored daily for clinical signs of disease such as inappetence, lethargy and lameness.

Vaccination. The chimeric vaccine used in this study was Suvaxyn® PCV2 (Fort Dodge Animal Health, Inc., serial number: 1861229A). The subunit vaccine was Ingelvac® CircoFLEX™ (Boehringer Ingelheim Vetmedica, serial number: 309-136). Each of the pigs in the vaccinated groups received 2 ml of Suvaxyn® PCV2 or 1 ml of Ingelvac® CircoFLEX™ of the vaccine intramuscularly into the right neck using a 0.77 mm 22 GA needle. Vaccination was done at d5 or d21.

PCV2b, PPV, and PRRSV inoculation. All pigs, excluding the negative control group, were inoculated at d49 with PCV2b, PPV, and PRRSV.

PCV2 inoculation. The PCV2 inoculum consisted of a PCV2b isolate NC-16845 (28) that was propagated on PK-15 cells to a titer of $10^{4.5}$ 50% tissue culture infectious dose (TCID₅₀) per ml. PCV2 inoculation was done by administering 1 ml of the inoculum intramuscularly into the right neck and 2 ml by slowly dripping the inoculum intranasally (1 ml per nostril) while the pig was held in the upright position.

PPV inoculation. The PPV inoculum consisted of a tissue homogenate containing strain NADL-8 with a titer of $10^{6.0}$ TCID₅₀ per ml (21). PPV inoculation was done by slowly dripping 1 ml of inoculum intranasally while the pig was held in the upright position.

PRRSV inoculation. The PRRSV inoculum consisted of a PRRSV isolate ATCC VR2385 (12). The PRRSV was propagated on MARC-145 cells to the 7th passage with a titer of $10^{5.0}$ TCID₅₀ per ml. PRRSV inoculation was done by slowly dripping 2.5 ml of inoculum intranasally while the pig was held in the upright position.

Serology. *PCV2.* All pig sera, from d21 to d70, were tested for anti-PCV2 antibodies using a PCV2 open-reading-frame (ORF2) based ELISA as previously described (24). A sample-to-positive (S/P) ratio of greater than or equal to 0.2 was considered positive. A fluorescent focus neutralization (FFN) assay was performed on serum samples collected on d49 for the detection of neutralizing antibodies using a previously described method (34).

PPV. The presence of anti-PPV IgG antibodies were detected in serum from d49 and d70 via hemagglutination inhibition (HI) assay, as previously described (22).

PRRSV. All pig sera from d49 and d70 were tested for anti-PRRSV antibodies by ELISA (IDEXX® PRRS X3Ab test, IDEXX Laboratories Inc., Westbrook, MA) according to the manufacturer's instructions. An S/P ratio of 0.4 was used as the minimum positive cutoff value.

Quantitative real-time PCR assays. *Total nucleic acid extraction.* All d49, d56, d63 and d70 serum samples were extracted using the total nucleic acid extraction kit, MagMAX™ viral isolation kit (Applied Biosystems, Foster City, CA) with the KingFisher Flex magnetic particle processor extraction system (Thermo Fisher Scientific, Waltham, MA).

PCV2. PCV2 viremia was determined by the detection of the presence and quantity of viral DNA in serum from all pigs on d49, d56, d63 and d70 via quantitative PCR using the same primers and probes as previously described (37). This was done in a 7500 Fast Real-Time PCR system (ABI, Foster City, CA). A final 25 µl volume containing 2.5 µl of extracted DNA was processed in the following cycles: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, 40 cycles of 30 sec at 55°C, and 40 cycles of 1 min at 60°C.

PPV. Viremia for PPV was determined by detection of the presence and quantity of PPV DNA in serum samples collected on d49, d56, d63 and d70 via quantitative real-time PCR as previously described (37). The final volume used in the reaction was 25 µl, which consisted of 12.5 µl of commercially available master mix (TaqMan® Universal PCR master mix, PE Applied Bio systems), 2.5 µl of DNA from either sample extraction or standard, 0.4 µM of each primer, and 0.2 µM of probe. The reaction consisted of the following cycles: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, and 40 cycles of 1 min at 60°C.

PRRSV. Quantitative real-time RT PCR for PRRSV viremia was processed on serum samples collected on d56, d63 and d70 using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) as previously described (37). PRRSV RNA presence and quantity were identified with real-time RT PCR utilizing TaqMan® NA and EA PRRSV Reagents (Applied Biosystems) with a final volume of 25 µl containing 12 µl of 2x multiplex RT-PCR buffer, 2.5 µl of 10x PRRSV primer probe mix, 1.25 µl of 20x multiplex enzyme mix, 0.75 µl of nuclease-free water, and 8 µl of either PRRSV RNA from the previous extraction or standard. The reaction consisted of

the following cycles: one cycle of 10 min at 45°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, and 40 cycles of 70 sec at 60°C.

Necropsy. All pigs were humanely euthanized with an overdose of pentobarbital sodium (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) at d70. The total amount of macroscopic lung lesions was estimated and scored (0-100% of the lung affected) as previously described (13). Sections of lung, heart, liver, lymph nodes (tracheobronchial, superficial inguinal, external iliac, mediastinal, and mesenteric lymph nodes), spleen, kidney, ileum, colon, tonsil and thymus were collected and placed into 10% neutral buffered formalin and routinely processed for histological examination.

Histopathology and immunohistochemistry. Microscopic examination of tissues was done by a veterinary pathologist who was blinded to the treatment groups. Lung sections were scored for presence and severity of interstitial pneumonia ranging from 0 to 6 (0=normal; 6=severe diffuse) (13). Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0=none to 3=severe. Lymphoid tissues including lymph nodes, tonsil, and spleen were evaluated for the presence of lymphoid depletion ranging from 0=normal to 3=severe lymphoid depletion with histiocytic replacement of follicles ranging from 0=normal to 3=severe (30).

Immunohistochemistry (IHC) for PCV2 was performed on formalin- fixed, paraffin-embedded tissue sections using a rabbit monoclonal antibody as previously described (39). Tissues evaluated include tonsil, spleen, lymph nodes (mesenteric, mediastinal, tracheobronchial, external inguinal and sub iliac) and thymus. PCV2-antigen scoring was done in a blinded fashion and scores ranged from 0=no signal to 3=more than 50% of the lymphoid follicles contain cells with PCV2-antigen staining (30).

The overall PCV2-associated lesions were scored as previously described (30). A combined scoring system for each lymphoid tissue that ranged from 0 to 9 (lymphoid depletion score 0-3; histiocytic replacement score 0-3; PCV2-IHC score 0-3) was used. The scores (lesions and PCV2-IHC) of the seven lymphoid tissues ([lymph node pool] × 5, spleen, and tonsil) were added together and divided by 7. The lymph node pool consisted of one section each of tracheobronchial, superficial inguinal, external iliac, mediastinal, and mesenteric lymph nodes. Pigs were grouped into four categories based on overall microscopic lymphoid lesion scores: normal (score=0), mild (score=1-3), moderate (score=4-6) and severe (score=7-9). A pig was diagnosed with PCVAD if the mean lymphoid microscopic lesion severity score was severe (score of 7-9). The mean group overall lymphoid score was calculated and compared between groups.

Statistical analysis. The data was statistically analyzed by performing a one-way analysis of variance (ANOVA) with JMP® software version 9.0.0 (SAS Institute, Cary, NC). The significance level was $p < 0.05$ followed by pairwise testing using the Tukey-Kramer adjustment to identify the groups that were different. All real-time PCR data were \log_{10} transformed prior to analysis. Percentage of reduction of PCV2 viremia in vaccinated groups compared to the non-vaccinated positive control group was done by measuring $100 - [(100 \times \text{the mean } \log_{10} \text{ genomic copies per ml serum in vaccinated animals}) / (\text{mean } \log_{10} \text{ genomic copies per ml serum in positive control animals})]$. Non-repeated measures such as histopathology data were assessed using non-parametric Kruskal-Wallis ANOVA. If a non-parametric ANOVA test was significant ($p < 0.05$), then Wilcoxon tests were used to assess the differences of pairs of groups. Differences in incidence were evaluated by using Fisher's exact test.

Results

Clinical disease. After challenge, triple-infected pigs developed mild-to-severe respiratory disease characterized by sneezing, increased respiratory rates and clear nasal discharge. A portion of the pigs also became lethargic. There were no significant differences between challenged groups.

Seroconversion to PCV2b, PPV and PRRSV. PCV2. The negative control pigs remained seronegative until termination of the study. Both of the d5 vaccinated groups had significantly ($p<0.05$) higher mean group S/P ratios than the d21 vaccinated groups up until d35 of the study. Vaccination with the chimeric product resulted in significantly ($p<0.05$) higher anti PCV2 IgG levels from d35 until d56 regardless of the day of vaccination. At d21, anti-PCV2 IgG was detected in 25% (2/8) of the *Subunit-d5* pigs and 87.5% (7/8) of the *Chimeric-d5* pigs. The prevalence of seropositive pigs was 100% at d28 for the d5 vaccinated pigs and 18.8% (3/16) for the d21 vaccinated pigs. All pigs in these groups were seropositive for PCV2 by d42.

Regardless of timing of vaccination, the chimeric vaccine was capable of inducing significantly ($p<0.05$) higher levels of broadly neutralizing antibodies at d49 than the subunit vaccine with mean group \log_{10} titers of 2.38 ± 0.18 for the chimeric vaccine compared to 1.82 ± 0.12 for the subunit vaccine. Positive control pigs started to seroconvert by d63 (62.5%; 5/8 pigs) and by d70 75% (6/8) of the pigs in this group had detectable anti-PCV2-IgG.

PPV. All groups were negative for anti-PPV antibodies on d49 and the non-challenged negative controls remained negative until d70. All pigs challenged with PPV seroconverted by d70; however, 2/8 positive control pigs had noticeably lower titers compared to all other pigs (1:2048). Overall, the mean group PPV titers of the PPV challenged were not different among treated groups (data not shown).

PRRSV. All pigs in all groups were negative for anti-PRRSV IgG on d49 and the non-challenged negative controls remained negative until d70. The majority of the pigs challenged with PRRSV seroconverted by d70 with the exception 2/8 positive control pigs. Overall, the mean group anti PRRSV IgG S/P ratios of the PRRSV challenged were not different among groups (data not shown).

PCV2, PPV and PRRSV viremia. PCV2. All pigs were negative for PCV2 DNA at the day of challenge and the negative control pigs remained negative for PCV2 DNA in serum until termination of the study at d70. The prevalence and the \log_{10} mean group amount of PCV2 DNA in the challenged groups are summarized in Table 1. All vaccinated groups had significantly ($p<0.05$) lower amounts of PCV2 DNA in serum compared to the positive control group. When the data were divided based on age of vaccination or product, no evidence of an effect of age or product was seen (data not shown). After challenge, PCV2 viremia was reduced by 75.4% to 100% in the vaccinated groups compared to the positive control group.

TABLE 1. Prevalence and mean group amount (\pm SE) of \log_{10} PCV2 DNA in pigs challenged with PCV2 on day of age (d) 49.

Group	d56	d63	d70
<i>Subunit-d21</i>	1/8 (0.7 \pm 0.7) ^{A*}	3/8 (1.6 \pm 0.8) ^{A,B}	1/8 (0.5 \pm 0.5) ^A
<i>Subunit-d5</i>	0/8 (0.0) ^A	4/8 (2.3 \pm 0.9) ^A	3/8 (1.5 \pm 0.7) ^A
<i>Chimeric-d21</i>	1/8 (0.6 \pm 0.6) ^A	0/8 (0.0) ^B	0/8 (0.0) ^A
<i>Chimeric-d5</i>	0/8 (0.0) ^A	0/8 (0.0) ^B	0/8 (0.0) ^A
Positive controls	7/8 (4.1 \pm 0.6) ^B	8/8 (7.1 \pm 0.3) ^C	8/8 (6.1 \pm 0.6) ^B

* Different superscripts (^{A,B,C}) within a column indicate significantly ($p<0.05$) amounts of group mean PCV2 DNA.

PPV. All pigs were negative for PPV DNA at the day of challenge and the negative control pigs remained negative until the termination of the study. The prevalence of PPV DNA positive pigs at d56 was 100% for *Subunit-d5* and *Subunit-d21* and it was 88.9% (7/8) for *Chimeric-d5*, *Chimeric-d21* and the positive control groups. The overall prevalence of PPV DNA positive animals was 68.8% (33/48) by d63 and 20.8% (10/48) by d70 with no significant differences among groups.

PRRSV. All pigs were negative for PRRSV RNA at the day of challenge and the negative control pigs remained PCR negative throughout the study. PRRSV RNA was detected in all PRRSV challenged pigs on d56, d63 and d70 without significant differences in the mean group PRRSV RNA levels among the challenged groups.

Gross lesions. There were no visible gross lesions in the non-infected control pigs. A portion of the triple-challenged pigs, regardless of vaccination status, had moderate-to-severe mottled tan colored, consolidated areas of lung tissue involving up to to 51% of the lung surface. A portion of the pigs had lymph nodes that were up to three times the normal size. There were no significant differences in gross lesions between challenged pigs.

Microscopic lesions and presence of PCV2 antigen in tissues. The majority of the pigs developed mild to severe interstitial pneumonia lesions characterized by thickening of alveolar septa by macrophages and lymphocytes and mild to severe type 2 pneumocyte hypertrophy and hyperplasia. The mean group interstitial pneumonia scores ranged from 3.0 ± 0.1 to 3.6 ± 0.4 in the triple-challenged groups and were significantly higher ($p<0.05$) compared to the negative control group (0.8 ± 0.1). Lymphoid lesions, if present, were characterized by mild-to-severe lymphoid depletion and mild-to-severe histiocytic replacement of lymphoid follicles.

The prevalence of PCV antigen and overall lymphoid lesion score for the different groups are summarized in Table 2. The majority of vaccinated pigs had no remarkable lesions and were considered normal. Individual vaccinated pigs (7/32) had an overall lesion score of 1 or 2. In the positive control group, 25% (2/8) of the pigs had microscopic lesions compatible with PCVAD associated with abundant amounts of PCV2 antigen and an overall lymphoid score of 9, 37.5% (3/8) of the pigs had moderate lymphoid lesions, and the remaining 37.5% (3/8) of the pigs had no to mild lymphoid lesions.

TABLE 2. Mean group overall lymphoid depletion score (\pm SE) and prevalence of PCV2 antigen in lymphoid tissues as determined by IHC.

Group	Overall lymphoid lesion score	Prevalence of PCV2 antigen
<i>Subunit-d21</i>	$0.11\pm 0.1^{A*}$	1/8
<i>Subunit-d5</i>	0.38 ± 0.2^A	2/8
<i>Chimeric-d21</i>	0.09 ± 0.09^A	0/8
<i>Chimeric-d5</i>	0.30 ± 0.2^A	2/8
Negative controls	0.0 ± 0.0^A	0/8
Positive controls	4.59 ± 1.1^B	7/8

*Significant differences among groups are indicated by different superscripts (^{A,B})

Discussion

The main objective of this study was to determine the efficacy of PCV2 vaccination at an earlier age than recommended by the vaccine manufacturers. Many research groups have studied the efficacy of commercial PCV2 vaccines in piglets pigs singularly infected with PCV2 (26, 27) or in pigs concurrently infected with multiple pathogens (29, 37). In all previous studies, vaccination was done per manufacturer and label instructions. To the authors' knowledge this is the first controlled experimental study to test the efficacy of commercial vaccines used in an off label manner in pigs less than 3 weeks of age which mimics what is commonly now done in the field. Many producers prefer to vaccinate with a single dose PCV2 product while processing piglets between 2 and 5 days of age. Early vaccination (before 7 days of age) has many potential benefits including maximizing efficacy in use of labor as the piglets only need to be handled once if PCV2 vaccinations can be done at the time when pigs are handled for other reasons (clipping tails and needle teeth, giving iron injections etc.). However, there is concern that the immune system may not be mature enough to effectively respond to the vaccinations potentially resulting in decreased vaccine efficacy and duration of immunity. To address the pro and cons of early vaccination, this study had piglets blocked by litter and randomly assigned to early vaccination (d5), regular vaccination (d21) or no vaccination (positive and negative controls). After challenge, PCV2 viremia and associated lesions were similarly reduced in all vaccinated pigs regardless of timing of vaccination indicating that d5 pigs are capable of mounting a protective immune response.

To determine if there were differences in efficacy of one vaccine over another, two different products were used side by side in this study: a chimeric and a subunit PCV2 vaccine. Several previous studies have been done to determine the efficacy of commercially available subunit PCV2 vaccines (9, 38) and chimeric PCV2 vaccines (8). In these studies, vaccinated animals have been shown to have strong antibody development associated with decreased PCV2 viremia after challenge. Similarly, in our study the vaccinated animals, regardless of the type of PCV2 vaccine used, all developed a detectable antibody response and protective immunity as evidenced by significantly decreased PCV2 viremia and decreased incidence and severity of lesions compared to the positive control group. Interestingly, and similar to a previous study using single dose vaccination (37), vaccination with the chimeric product was associated with production of a stronger IgG response than with the subunit vaccine and with a numerically reduction in viremia.

PRRSV and PPV are both common pathogens in commercial swine herds and both can contribute to PCVAD outbreaks (6, 9, 32, 33). Several studies have been conducted in dual or triple challenge models (1, 2, 30, 37). Both PRRSV RNA and PPV DNA were detected in vaccinated and positive control animals that were part of a PCV2 challenge study; however, no difference were noted between groups (37). Similarly, in the present study, PRRSV RNA and PPV DNA were both detected by d56 (seven days after challenge) without differences among groups regardless of PCV2 vaccination status.

To reduce the risk of cross contamination between rooms, collection of some clinical parameters such as weight gain were not evaluated in this study. However, when assessing microscopic lesions and PCV2 antigen levels in tissues, two of eight positive control pigs were identified as having lesions compatible with PCVAD. These two pigs did not seroconvert to PRRSV and had only a weak serological response to PPV further indicating that they were immune suppressed which is typical for pigs with PCVAD (25). Three additional positive controls pigs had overall moderate microscopic lesions indicating that the triple challenge used was successful. As vaccinated pigs were protected from development of PCV2 associated lesions and this was independent of timing of vaccination this is further indication that both 5d and 21d vaccination protocols with either vaccine were effective. The immune system of very young pigs is apparently capable of mounting a protective immune response against PCV2. The current study was done in PCV2 naïve pigs; however, under field conditions most pigs will be seropositive due to the ubiquitous nature of PCV2 and high levels of PCV2 antibody in colostrum. Therefore, future studies should investigate the effect of passively acquired antibodies on early vaccination.

In this study, all vaccines and protocols were capable of significantly reducing PCV2 viremia and PCV2 associated lesions in tissues compared to the positive control group with negligible differences between products and vaccination times. Regardless of the age at vaccination, the pigs were capable of developing a protective immune response in PCV2 naïve pigs.

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