CLINICAL AND VACCINE IMMUNOLOGY, Nov. 2011, p. 1865–1871 Vol. 18, No. 11
1556-6811/11/$12.00 doi:10.1128/CVI.05318-11

Studies on Porcine Circovirus Type 2 Vaccination of 5-Day-Old Piglets▽

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Received 25 July 2011/Returned for modification 22 August 2011/Accepted 12 September 2011

Porcine circovirus type 2 (PCV2) vaccines have become widely used since they became available in 2006. It is not uncommon for producers to use PCV2 vaccines in pigs younger than what is approved 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 days of age. Forty-eight PCV2-naïve piglets were randomly divided into six groups of eight pigs each. Vaccination was done at day 5 or day 21, followed by triple challenge with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV) at day 49. Vaccinated pigs seroconverted to PCV2 approximately 14 days postvaccination and had a detectable neutralizing antibody response by 21 days postvaccination regardless of age at vaccination. At day 49, the pigs vaccinated with the chimeric vaccine had significantly higher levels of neutralizing antibodies than the pigs vaccinated with the subunit vaccine. After challenge, vaccinated pigs had significantly decreased levels of PCV2 viremia and a decreased prevalence and severity of microscopic lesions compared to the positive-control group, which had severe lymphoid lesions associated with abundant PCV2 antigen, compatible with PCV-associated disease. The results of this study indicate that, under the conditions of this study, vaccination of PCV2-naïve pigs at day 5 or day 21 resulted in development of a detectable humoral immune response and provided reduction or complete protection against PCV2 viremia and PCV2-associated lesions after triple challenge with PCV2, PPV, and PRRSV.

Porcine circovirus (PCV) is a circular, single-stranded, nonenveloped DNA virus (46) that can be separated into two main types: PCV type 1 (PCV1) and PCV type 2 (PCV2). PCV1 is not associated with disease or lesions in pigs and is commonly considered nonpathogenic (47). PCV2 is linked with a variety of clinical disease manifestations collectively referred to as PCV-associated disease (PCVAD), including systemic disease or postweaning multisystemic wasting syndrome (PMWS) (16), respiratory disease (17), and enteric disease (20) in growing pigs.

PCV2 is prevalent worldwide, and most herds are seropositive (5, 6, 47). From 2005 to 2006, PCVAD became increasingly problematic in North America, leading to high production losses for producers (19). Aggressive vaccination programs initiated in 2006 have substantially decreased the prevalence and severity of PCVAD (14, 21).

U.S. pork producers now have several choices of approved commercial vaccines. Two of the commercial PCV2 vaccines commonly used in the United States are a subunit vaccine and a chimeric vaccine. One of the subunit vaccines (Ingelvac CircoFLEX; Boehringer Ingelheim Vetmedica) is licensed for use in pigs at 3 weeks of age or older, provides protection beginning 2 weeks postvaccination, and has at least a 17-week duration of immunity. The inactivated chimeric PCV2 vaccine (formerly Suvaxyn PCV2 from Fort Dodge Animal Health, Inc., and now reformulated as Fostera PCV from Pfizer Animal Health, Inc.) is also licensed for use in pigs 3 weeks of age or older. According to the manufacturer, this product provides protection against PCV2 challenge 3 weeks (two-dose application) or 6 weeks (one-dose application) postvaccination for up to 4 months duration. This product was voluntarily removed from the market in May 2010 due to concerns regarding the inactivation process (13) and was reintroduced to the market in August 2011.

In the field, coinfections heavily influence the severity and outcome of PCVAD. Some of the most severe field case reports of PCVAD describe coinfection of pigs with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV) (7, 8). PPV has been shown to cause stillbirths and mummification in breeding herds but is generally considered nonpathogenic in growing pigs (22); however, when pigs are coinfected with PCV2 and PPV, this can lead to severe PCVAD in a portion of the pigs (1, 8, 22). PRRSV has become endemic and is known to cause abortions in the breeding herd and pneumonia in growing pigs (39). When found combined with PCV2 in the field or when pigs are experimentally coinfected with PRRSV and PCV2, disease and lesions are often quite severe (2, 18, 40).

A common concern when evaluating a vaccination program, besides the efficacy of the vaccine, is the appropriate timing of vaccination to provide maximal protection for the pig and convenience of use for the pork producer. Vaccines are commonly labeled for use at day 21 or older. Many pig farm

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▽ Published ahead of print on 21 September 2011.

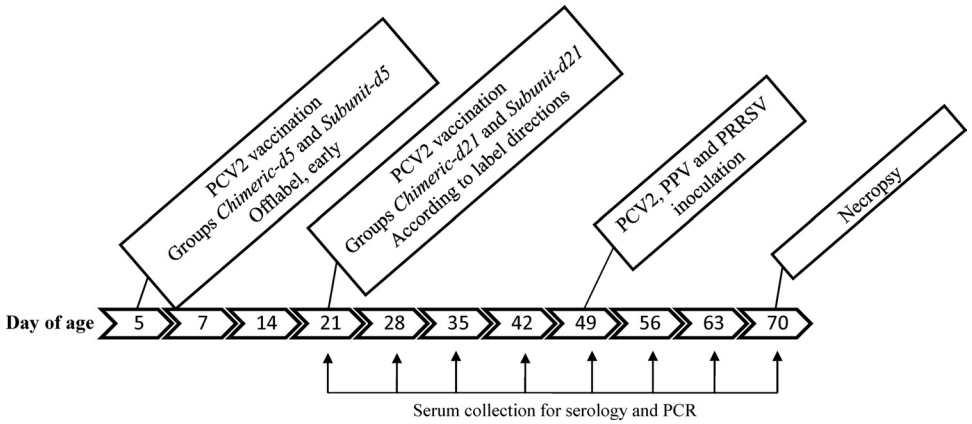

FIG. 1. Experimental design. All serum samples collected were tested for the presence of PCV2 antibody. Samples from day of age 49, 56, 63, and 70 were tested for the presence and amount of PCV2 DNA and PPV DNA. Samples from day 56, day 63, and day 70 were tested for the presence and amount of PRRSV RNA. Samples from day 49 and day 70 were tested serologically for PRRSV and PPV.

managers prefer to vaccinate pigs at day 2 to day 5, which is when they are handling piglets for other reasons. There are concerns, ongoing discussions, and debate over whether the pig has a sufficiently mature immune system at this age and if passively acquired antibodies interfere with vaccination. Therefore, if vaccination against pathogens such as PCV2 is proven to be effective in pigs less than 1 week of age, this ultimately could lead to substantial changes in vaccination protocols on many farms.

The objective of this study was to determine the efficacy of two commercial PCV2 vaccines, an inactivated chimeric vaccine and a subunit vaccine, at day 5 and day 21, in a triple challenge model with PCV2, PPV, and PRRSV. The triple challenge model was used to mimic field conditions where coinfections with PCV2, PPV, and PRRSV are commonly observed (7, 8, 35, 36).

MATERIALS AND METHODS

Animals and housing. Forty-eight conventional cross-bred 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 Isolation 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 inactivated 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. Each room had 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 day 5 or day 21. 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. The 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. The timeline of the experiment is summarized in Fig. 1. At day 5, 16 pigs were vaccinated with one of two PCV2 vaccines: an inactivated chimeric vaccine (chimeric-d5) or a viral subunit vaccine (subunit-d5). Similarly at day 21, 16 pigs were vaccinated with the inactivated 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 (Becton Dickinson vacutainer; 8.5 ml) and centrifuged at 2,000 $\times$ *g* for 10 min at 4°C, and the serum was separated into two aliquots and stored at −80°C until testing. All pigs, except for the negative-control group, were inoculated with PPV, PRRSV, and PCV2b at day 49, and all pigs were euthanized for necropsy at day 70.

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

Vaccination. The inactivated chimeric vaccine used in this study was Suvaxyn PCV2 (serial number 1861229A; Fort Dodge Animal Health, Inc.). The subunit vaccine was Ingelvac CircoFLEX (serial number 309-136; Boehringer Ingelheim Vetmedica). Each of the pigs in the vaccinated groups received 2 ml of Suvaxyn PCV2 or 1 ml of Ingelvac CircoFLEX vaccine intramuscularly into the right neck via a 0.77-mm 22-gauge needle. Vaccination was done at day 5 or day 21.

PCV2b, PPV, and PRRSV inoculation. All pigs, excluding the negative-control group, were inoculated at day 49 with PCV2b, PPV, and PRRSV.

PCV2 inoculation. The PCV2 inoculum consisted of PCV2b isolate NC-16845 (32), which was propagated on PK-15 cells to a titer of $10^{4.5}$ 50% tissue culture infective doses ($TCID_{50}$) per ml. PCV2 inoculation was done by administering 1 ml of the inoculum intramuscularly into the right neck and slowly dripping 2 ml of 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 at a titer of $10^{6.0}$ $TCID_{50}$ per ml (25). 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 PRRSV isolate ATCC VR2385 (15). PRRSV was propagated on MARC-145 cells to the seventh passage at a titer of $10^{5.0}$ $TCID_{50}$ 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. (i) PCV2. All pig sera, from day 21 to day 70, were tested for anti-PCV2 antibodies by a PCV2 capsid protein-based enzyme-linked immunosorbent assay (ELISA) as previously described (28). 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 21 days after vaccination for all vaccinated pigs and at the day of challenge (day 49) for all pigs for the detection of neutralizing antibodies, using a previously described method (37).

(ii) PPV. The anti-PPV IgG antibodies were detected in serum from day 49 and day 70 via a hemagglutination inhibition (HI) assay, as previously described (26).

(iii) PRRSV. All pig sera from day 49 and day 70 were tested for anti-PRRSV antibodies by ELISA (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. (i) Total nucleic acid extraction. All day 49, day 56, day 63, and day 70 serum samples were extracted using a 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).

(ii) PCV2. PCV2 viremia was determined by the detection of the presence and amount of viral DNA in serum samples from all pigs on day 49, day 56, day 63, and day 70 via quantitative PCR using the same primers and probes as previously described (42). This was done in a 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA). A final 25-μl volume containing 2.5 μl of extracted DNA was processed under the following thermocycler conditions: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

(iii) PPV. Viremia for PPV was determined by detection of the presence and amount of PPV DNA in serum samples collected on day 49, day 56, day 63, and day 70 via quantitative real-time PCR as previously described (42). The final volume of the reaction mixture was 25 μl, which consisted of 12.5 μl of commercially available master mix (TaqMan Universal PCR master mix; PE Applied Biosystems), 2.5 μl of DNA from either sample extraction or standard, 1 μl (0.4 μM) of each primer, and 0.5 μl (0.2 μM) of the probe. The thermocycler conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

(iv) PRRSV. Quantitative real-time reverse transcription-PCR (RT-PCR) for PRRSV viremia was performed on serum samples collected on day 56, day 63, and day 70 using the TaqMan NA and EU PRRSV reagents (Applied Biosystem) as previously described (42). PRRSV RNA presence and quantity were identified with real-time RT-PCR by utilizing TaqMan NA and EA PRRSV reagents (Applied Biosystems) with a final volume of 25 μl, containing 12 μl of 2× multiplex RT-PCR buffer, 2.5 μl of 10× PRRSV primer probe mix, 1.25 μl of 20× multiplex enzyme mix, 0.75 μl of nuclease-free water, and 8 μl of either PRRSV RNA from the previous extraction or standards. The thermocycler conditions were as follows: 10 min at 45°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 70 s at 60°C.

Necropsy. All pigs were humanely euthanized with an overdose of pentobarbital sodium (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI) at day 70. The total amount of macroscopic lung lesions was estimated and scored (0 to 100% of the lung affected) as previously described (15). The sizes of lymph nodes (score range from 0 to 3: 0 [normal], 1 [two times the normal size], 2 [three times the normal size], and 3 [four times the normal size]) were estimated as described previously (29). Sections of lung, heart, liver, lymph nodes (tracheobronchial, superficial inguinal, external iliac, mediastinal, and mesenteric), spleen, kidney, ileum, colon, tonsil, and thymus were collected, 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, with scores ranging from 0 to 6 (0 [normal]; 6 [severe diffuse]) (15). Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored on a scale of 0 (none) to 3 (severe). Lymphoid tissues, including lymph nodes, tonsil, and spleen, were evaluated for the presence of lymphoid depletion, with scores ranging from 0 (normal) to 3 (severe lymphoid depletion) and scores for histiocytic replacement of follicles ranging from 0 (normal) to 3 (severe) (34).

Immunohistochemistry (IHC) for PCV2 was performed on formalin-fixed, paraffin-embedded tissue sections by using a rabbit polyclonal antibody as previously described (45). Tissues evaluated included tonsil, spleen, lymph nodes (mesenteric, mediastinal, tracheobronchial, external inguinal, and subiliac), and thymus. PCV2 antigen scoring was performed in a blinded fashion, and scores ranged from 0 (no signal) to 3 (more than 50% of lymphoid follicles containing cells with PCV2 antigen staining) (34).

The overall PCV2-associated lesion scores were determined as previously described (34). A combined scoring system for each lymphoid tissue that ranged from 0 to 9 (lymphoid depletion score, 0 to 3; histiocytic replacement score, 0 to 3; PCV2 IHC score, 0 to 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 score: normal (score of 0), mild (score of 1 to 3), moderate (score of 4 to 6), and severe (score of 7 to 9). A pig was diagnosed with PCVAD if the mean lymphoid microscopic lesion severity score was severe (score of 7 to 9). The mean group overall lymphoid score was calculated and compared between groups.

Statistical analysis. The data were 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. The percentage of reduction of PCV2 viremia in vaccinated groups compared to the nonvaccinated positive-control group was calculated as follows: $100 - [(100 \times \text{mean } \log_{10} \text{ genomic copies per ml of serum in vaccinated animals})/(\text{mean } \log_{10} \text{ genomic copies per ml of serum in positive-control animals})]$. Nonrepeated measures, such as histopathology data, were assessed using a nonparametric Kruskal-Wallis ANOVA. If a nonparametric 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-challenged pigs in all groups developed mild to severe respiratory disease characterized by sneezing, increased respiratory rates, and clear nasal discharge. A portion of the triple-challenged pigs also became lethargic.

Seroconversion against PCV2, PPV, and PRRSV. (i) PCV2. The negative-control pigs remained seronegative until termination of the study (Fig. 2A). Seroconversion to PCV2 in the vaccinated groups was similar for the day 5 (Fig. 2B) and day 21 (Fig. 2C) groups. By 14 days postvaccination, 2/8 subunit-d5, 3/8 subunit-d21, 7/8 chimeric-d5, and 8/8 chimeric-d21 animals had seroconverted; by 21 days after vaccination all vaccinated pigs except 2/8 subunit-d21 animals had seroconverted; by 28 days after vaccination all vaccinated pigs were seropositive for PCV2. There was a trend to lower levels of detectable anti-PCV2 IgG in pigs vaccinated with the subunit vaccine compared to those vaccinated with the chimeric vaccine, and this was independent of age of vaccination (Fig. 2B and C). The mean amounts of neutralizing antibody levels 21 days postvaccination were similar in pigs vaccinated at day 5 (mean group $\log_{10}$ titers of 1.84 ± 0.16) (± standard error [SE]) and day 21 (1.56 ± 0.12); however, there was a significant difference when the data were analyzed by product (2.01 ± 0.14 for the chimeric vaccine and 1.39 ± 0.11 for the subunit vaccine).

As expected, when the data were evaluated by day of age rather than by days after vaccination, vaccination at day 5 resulted in significantly ($P < 0.05$) higher anti-PCV2 IgG levels from day 21 until day 42; however, there were no differences between the day 5- and day 21-vaccinated groups thereafter (Fig. 2A). At day 21, 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 day 28 for the day 5-vaccinated pigs and 18.8% (3/16) for the day 21-vaccinated pigs. All pigs in these groups were seropositive for PCV2 by day 42. Regardless of timing of vaccination, the chimeric vaccine induced significantly ($P < 0.05$) higher levels of neutralizing antibodies at day 49 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 day 63 (62.5%; 5/8 pigs) and day 70 (75%; 6/8 pigs) as detected by ELISA.

(ii) PPV. All groups were negative for anti-PPV antibodies on the day of challenge (day 49), and the nonchallenged negative controls remained negative until day 70. All pigs challenged with PPV seroconverted by day 70; however, 2/8 positive-control pigs had noticeably lower titers (1:2,048) than all other pigs (1:4,096 to >16,384). Overall, the mean group PPV titers of the PPV-challenged animals were not different among treated groups (data not shown).

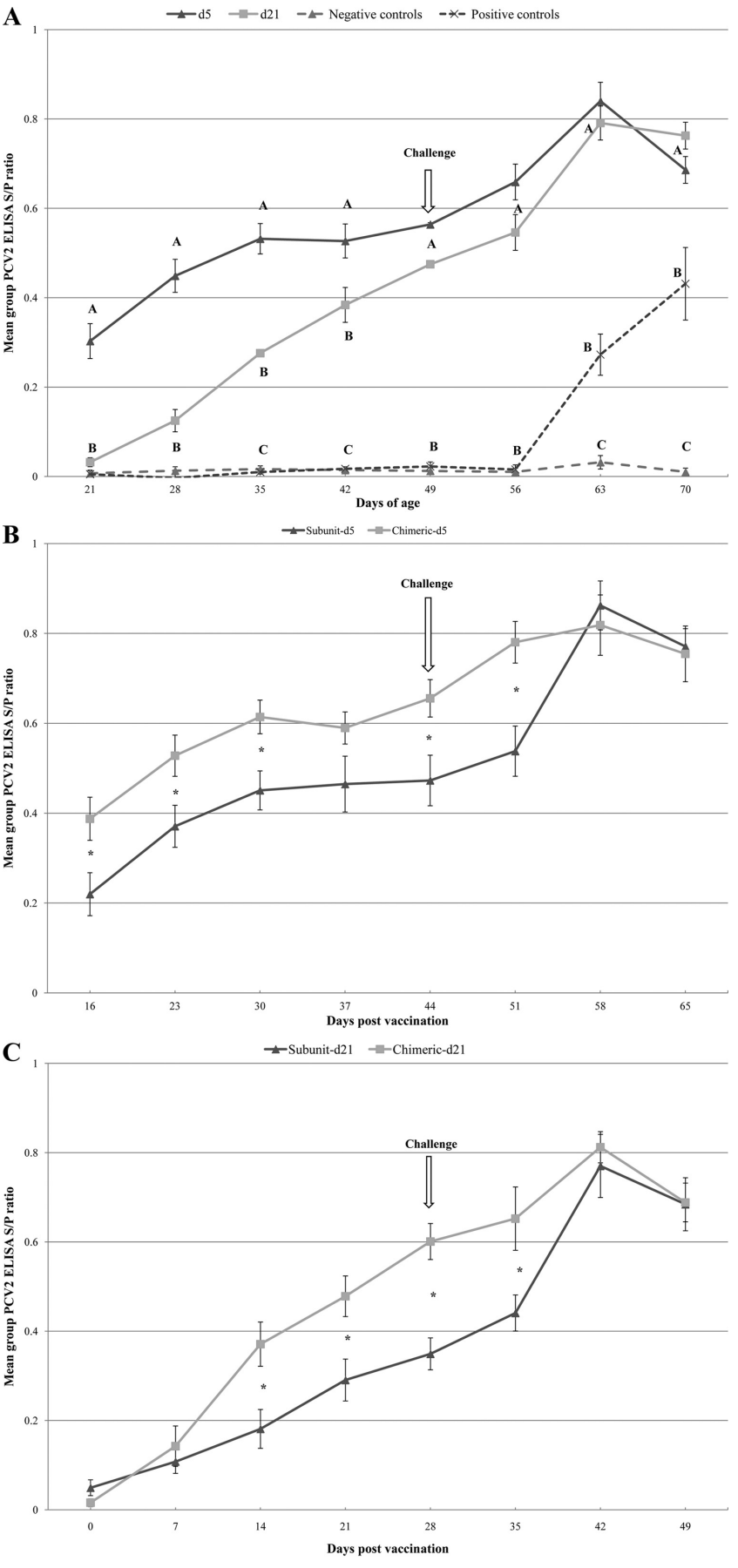
A
d5
d21
Negative controls
Positive controls
Challenge
Mean group PCV2 ELISA S/P ratio
Days of age
B
Subunit-d5
Chimeric-d5
Challenge
Mean group PCV2 ELISA S/P ratio
Days post vaccination
C
Subunit-d21
Chimeric-d21
Challenge
Mean group PCV2 ELISA S/P ratio
Days post vaccination

TABLE 1. Prevalence and mean $\log_{10}$ PCV2 DNA in pigs challenged with PCV2 at age 49 days

Group	Prevalence (mean level ± SE)[a] of $\log_{10}$ PCV2 DNA on day:		
	56	63	70
Subunit-d21	1/8 (0.7 ± 0.7)^{A}	3/8 (1.6 ± 0.8)^{A,B}	1/8 (0.5 ± 0.5)^{A}
Subunit-d5	0/8 (0.0)^{A}	4/8 (2.3 ± 0.9)^{A}	3/8 (1.5 ± 0.7)^{A}
Chimeric-d21	1/8 (0.6 ± 0.6)^{A}	0/8 (0.0)^{B}	0/8 (0.0)^{A}
Chimeric-d5	0/8 (0.0)^{A}	0/8 (0.0)^{B}	0/8 (0.0)^{A}
Positive controls	7/8 (4.1 ± 0.6)^{B}	8/8 (7.1 ± 0.3)^{C}	8/8 (6.1 ± 0.6)^{B}

[a] Different superscript capital letters (A, B, and C) within a column indicate significantly ($P < 0.05$) different amounts of group mean PCV2 DNA.

TABLE 2. Lymphoid depletion score and prevalence of PCV2 antigen in lymphoid tissues as determined by IHC

Group	Overall lymphoid lesion score (mean ± SE)[a]	Prevalence of PCV2 antigen
Subunit-d21	0.11 ± 0.1^{A}	1/8
Subunit-d5	0.38 ± 0.2^{A}	2/8
Chimeric-d21	0.09 ± 0.09^{A}	0/8
Chimeric-d5	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

[a] Significant differences among groups are indicated by different superscript capital letters (A and B).

(iii) PRRSV. All pigs in all groups were negative for anti-PRRSV IgG on the day of challenge (day 49), and the nonchallenged negative controls remained negative until day 70. The majority of the pigs challenged with PRRSV seroconverted by day 70, with the exception of 2/8 positive-control pigs. Overall, the mean group anti-PRRSV IgG S/P ratios of the PRRSV-challenged pigs were not different among groups (data not shown).

PCV2, PPV, and PRRSV viremia. (i) PCV2. All pigs were negative for PCV2 DNA at the day of challenge (day 49), and the negative-control pigs remained negative for PCV2 DNA in serum until termination of the study at day 70. 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 ($P < 0.05$) smaller amounts of PCV2 DNA in serum than the positive-control group. When the data were divided based on age of vaccination, no evidence of an effect of age at vaccination on PCV2 viremia was seen. However, pigs vaccinated with the chimeric vaccine had significantly lower mean amounts of PCV2 genomic copies in serum samples on day 63 ($P = 0.021$) and day 70 ($P = 0.03$) than those vaccinated with the subunit vaccine. After challenge, PCV2 viremia was reduced by 75.4% to 100% in the vaccinated groups compared to the positive-control group.

(ii) PPV. All pigs were negative for PPV DNA at the day of challenge (day 49), and the negative-control pigs remained negative until the termination of the study. The prevalence of PPV DNA positive pigs at day 56 was 100% for subunit-d5 and subunit-d21, and it was 88.9% (7/8) for the chimeric-d5, chimeric-d21, and the positive-control groups. The overall prevalence rate of PPV DNA-positive animals was 68.8% (33/48) by day 63 and 20.8% (10/48) by day 70, with no significant differences among groups.

(iii) PRRSV. All pigs were negative for PRRSV RNA at the day of challenge (day 49), and the negative-control pigs remained PCR negative throughout the study. PRRSV RNA was detected in all PRRSV-challenged pigs on day 56, day 63, and day 70 without significant differences in the mean group PRRSV RNA levels among the challenged groups, regardless of vaccination status.

Gross lesions. There were no visible gross lesions in the noninfected 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 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 ($P < 0.05$) than for 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 rates of PCV2 antigen and overall lymphoid lesion scores 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; the remaining 37.5% (3/8) of the pigs had no to mild lymphoid lesions.

DISCUSSION

The main objective of this study was to determine the efficacy of PCV2 vaccination at an earlier age than recommended

FIG. 2. (A) Mean group PCV2 ELISA S/P ratios (± SE) on serum collected from piglets vaccinated at day of age 5 (d5) or 21 (d21) or nonvaccinated and challenged with PCV2, PPV, and PRRSV at day 49, which corresponds to 44 days after vaccination for day 5 piglets and 28 days after vaccination for day 21 piglets. An S/P ratio of 0.2 or greater was considered seropositive. Significant differences among groups on a certain day are indicated by different letters (A, B, and C). (B) Comparison of subunit-d5 and chimeric-d5 pigs at different days postvaccination. Significant differences among groups on a certain day are indicated by an asterisk. (C) Comparison of subunit-d21 and chimeric-d21 pigs at different days postvaccination. Significant differences among groups on a certain day are indicated by an asterisk.

by the vaccine manufacturers. Several research groups have studied the efficacy of commercial PCV2 vaccines in pigs singularly infected with PCV2 (30, 31) or in pigs concurrently infected with multiple pathogens (33, 42). In all previous studies, vaccination was done according to the manufacturer's label instructions. To our knowledge, this is the first controlled experimental study to test the efficacy of commercial vaccines used at less than 3 weeks of age in a manner not approved by the manufacturer; however, this regimen mimics what is commonly now done in the field in the United States. Many producers prefer to vaccinate with a single-dose PCV2 product while piglets are undergoing castration, iron shots, tail docking, and teeth clipping between 2 and 5 days of age. 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 evaluate the benefits and shortcomings of early vaccination, this study entailed use of piglets blocked by litter and randomly assigned to early vaccination (day 5), regular vaccination (day 21), or no-vaccination (positive- and negative-control) groups.

After challenge, PCV2 viremia and associated lesions were similarly reduced in all vaccinated pigs regardless of timing of vaccination, indicating that day 5 pigs are capable of mounting a protective immune response. Vaccinated pigs were protected from development of PCV2-associated lesions independent of timing of vaccination, further indicating that both day 5 and day 21 vaccination protocols with either vaccine were effective. The pig immune system is unique in many ways that may be responsible for its ability to develop protective immunity from early vaccination. These factors include the full-length complementarity-determining region 3 (CDR3) of the heavy chain of immunoglobulin (4), limited genetic combinatorial preimmune repertoire development (4), and the absence of true gene conversion sometimes seen in other species (44). The above-mentioned characteristics of the pig immune system combined with the results of this study demonstrate that the 5-day-old suckling pig is indeed capable of mounting a protective immune response against PCV2 challenge.

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 anti-PCV2 antibodies in colostrum. Interference with vaccination against swine influenza virus associated with the presence of passively acquired antibodies has been documented (3, 23, 27, 38); however, evidence of passive antibody interference with PCV2 vaccination has not been confirmed under experimental conditions (30). Furthermore, PCV2 vaccines have been highly effective in the field, and almost all pigs are seropositive to PCV2 at the time of PCV2 vaccination (9, 19, 21, 41). In experimental PCV2 challenge models, outcomes are often similar between vaccine treatment groups (11, 24), and conclusions often lack power. Passively acquired antibodies in many instances decrease PCV2 viremia and prevent the development of clinical disease under controlled experimental conditions. PCV2 viremia and expression of clinical disease are often the main outcomes used for vaccine efficacy comparisons; however, when using animals with maternally derived anti-PCV2 antibodies, a much larger sample size may be required to demonstrate differences. Although the antibody-negative status of the pigs in the current study did not necessarily mimic what occurs with the majority of pigs in the field, studies performed in PCV2 antibody-free and PCV2 virus-free pigs are an important first step to increasing our understanding of potential advantages and disadvantages of early vaccination regimens.

To determine if there were differences in the efficacy of one vaccine over another, two different products were used side by side in this study. Several previous studies had been performed to determine the efficacy of PCV2 subunit vaccines and chimeric PCV2 vaccines (10, 12, 43). In these studies, vaccinated animals were shown to have strong antibody responses 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 a decreased incidence and severity of lesions compared to the positive-control group. However, pigs vaccinated with the chimeric product had a stronger anti-PCV2 IgG response that was independent of age at vaccination and a lower prevalence of PCV2 viremic animals at day 63 and day 70 than pigs vaccinated with the subunit product. Moreover, and similar to a previous study using single-dose vaccination (42), vaccination with the chimeric product was associated with production of a stronger neutralizing antibody response than vaccination with the subunit vaccine.

In summary, under the conditions of this study, vaccination with chimeric or subunit PCV2 vaccines at 5 or 21 days of age induced a protective immune response in PCV2-naïve pigs as demonstrated by development of anti-PCV2 antibodies and reductions of PCV2 viremia and PCV2-associated lesions in a triple challenge model with PCV2, PPV, and PRRSV.

ACKNOWLEDGMENTS

This study was funded by the National Pork Board Pork Checkoff Dollars.

We thank Shayleen Schalk for assistance with the animal work.

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