Porcine Reproductive and Respiratory Syndrome Virus Infection at the Time of Porcine Circovirus Type 2 Vaccination Has No Impact on Vaccine Efficacy[▽]

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Several porcine circovirus type 2 (PCV2) vaccines are now commercially available and have been shown to be effective at decreasing the occurrence of porcine circovirus-associated disease (PCVAD). Many herds are coinfected with PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV). Some producers and veterinarians are concerned that if pigs are vaccinated for PCV2 at or near the time that they are typically infected with PRRSV, the efficacy of the PCV2 vaccine will be compromised. The impact of PRRSV on PCV2 vaccination is unclear and has not been investigated under controlled conditions. The objective of the present study was to determine whether the presence of PRRSV viremia has an effect on the efficacy of commercial PCV2 vaccinations. Three-week-old PCV2-negative conventional pigs with passively derived anti-PCV2 antibodies were either vaccinated with one of three commercial PCV2 vaccines or left nonvaccinated. A portion of the pigs were infected with PRRSV 1 week prior to PCV2 vaccination. To determine vaccine efficacy, a PCV2 challenge was conducted at 8 weeks of age. PCV2 vaccination, regardless of PRRSV infection status at the time of vaccination, was similarly effective in inducing an anti-PCV2 IgG response in the presence of maternally derived immunity and in protecting the pigs from PCV2 challenge, as determined by a reduction in the level of PCV2 viremia and a reduction in the prevalence and amount of PCV2 antigen in lymphoid tissues in vaccinated pigs compared to nonvaccinated pigs. The results indicate that acute PRRSV infection at the time of PCV2 vaccination has no adverse effect on PCV2 vaccine efficacy.

Porcine circovirus (PCV) type 2 (PCV2) is a single-stranded, circular, nonenveloped DNA virus with an icosahedral symmetry (6, 44). It has an ambisense genome of approximately 1.8 kb and belongs to the family *Circoviridae*, members of which are known to be very host specific and often associated with subclinical infections (36, 45). PCV-associated disease (PCVAD), first recognized in 1991 as postweaning multisystemic wasting syndrome (PMWS) (15), has spread throughout the global swine population. Besides the systemic presentation of PCV2 infection, other disease manifestations of PCVAD include respiratory disease, enteritis in grow-finisher pigs, reproductive failure, and porcine dermatitis and nephropathy syndrome (PDNS) (27). Lymphoid depletion and granulomatous inflammation in several organ systems are the hallmark lesions of PCVAD (41).

Pigs concurrently infected with PCV2 and other pathogens have a greater potential of developing clinical PCVAD (27). In a retrospective study in South Korea, 76.2% (80/105) of pigs suffering from porcine respiratory disease complex (PRDC) were found to be coinfected with PCV2 and various other pathogens, including porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), and porcine parvovirus (PPV) (18). In another investigation in the United States, PRRSV was present in 51.9% of 484 cases of systemic PCVAD and was the most frequent pathogen (34). In a cross-sectional study performed with 583 conventionally reared pigs of different ages, coinfections of PCV2 with PRRSV, SIV, and *Mycoplasma hyopneumoniae* had the greatest effect on the development of diseases in the early to late nursery phases (8). In a case-control study of pigs with and without PCVAD, pigs with concurrent PCV2 and PRRSV infections had a higher odds ratio of developing PCVAD (35).

PRRSV is a single-stranded, positive-sense, enveloped RNA virus that belongs to the family *Arteriviridae*, genus *Arterivirus* (4). PRRSV primarily infects the monocyte/macrophage cell line (11) via CD163 scavenger receptor and heparin sulfate and sialoadhesin receptors (10, 46), which are also involved in the primary defense of the innate immune system (9, 21). It causes reproductive failure in pregnant sows and respiratory disease in pigs of all ages and is also associated with neonatal diarrhea (1, 3, 23, 37). Under experimental conditions, with concurrent PRRSV and PCV2 infection in 1- to 2-day-old colostrum-deprived (CD) pigs (2), 5-week-old PCV2-seropositive pigs (38), or 3-week-old cesarean-derived colostrum-deprived (CD/CD) pigs (16), more severe clinical disease and lesions as well as enhanced replication and distribution of PCV2 were ob-

TABLE 1. Experimental design

Group designation	No. of pigs	PRRSV infection (2 wk)	PCV2 vaccine amt (ml)		Challenge (10 wk)
			3 wk	5 wk	
PRRSV-FDAH-PCV2	10	PRRSV	2		PCV2
FDAH-PCV2	10		2		PCV2
PRRSV-BIVI-PCV2	10	PRRSV	1		PCV2
BIVI-PCV2	9		1		PCV2
PRRSV-Intervet-PCV2	10	PRRSV	2	2	PCV2
Intervet-PCV2	10		2	2	PCV2
PRRSV	11	PRRSV			
PCV2	10				PCV2
PRRSV-PCV2	10	PRRSV			PCV2
Negative controls	9				

served compared to the conditions for pigs inoculated with PCV2 alone. These results suggest that immune modulation by PRRSV may be a key factor in the development of PCVAD.

PRRSV can be transmitted transplacentally, resulting in infected neonates, and PRRSV viremia may last for up to 154 days, with virus being detectable in tissues for up to 202 days (20). PRRSV seroconversion and viremia typically occur during the nursery phase of production. It is a common practice for veterinarians and producers to adjust the timing of administration (delay or move earlier) of common vaccinations, such as vaccination for *M. hyopneumoniae* (9), to avoid vaccine failure associated with PRRSV-induced immunosuppression.

At present, no information exists on the efficacy of PCV2 vaccination in PRRSV viremic pigs. The objective of this study was to determine the effect of PRRSV infection at the time of PCV2 vaccination on PCV2 vaccine efficacy. Three currently available commercial PCV2 vaccines were compared in the conventional pig model using pigs with passively acquired anti-PCV2 antibodies with or without experimental PRRSV infection.

MATERIALS AND METHODS

Animals and housing. Ninety-nine specific-pathogen-free (SPF) pigs were weaned at 2 weeks of age from high-health sows free of PRRSV and SIV. PCV2 vaccination was not used in the breeding herd. The dams of the piglets used in this study were not PCV2 viremic (negative for PCV2 DNA on serum evaluation) but were positive for PCV2 antibody, as determined by PCV2 enzyme-linked immunosorbent assay (ELISA) approximately 1 week before farrowing. Upon arrival at the research facility at Iowa State University, the pigs were housed in four separate rooms: the noninfected negative-control group and the group infected with PRRSV alone were housed in separate rooms, and all other pigs regardless of treatment group were randomly assigned to one of two rooms, which each contained four pens. The pens were 2 m by 2.3 m in size, and each one was equipped with one nipple drinker and a self feeder that contained a complete feed ration free of animal proteins (excluding whey) and antibiotics (Nature's Made; Heartland Coop, IA).

Experimental design. The 99 pigs were randomly divided into 10 groups of 9 to 11 pigs in each group after arrival at the Iowa State University research facility. The experimental design is summarized in Table 1. At 2 weeks of age, 51 out of 99 pigs were inoculated with PRRSV. At 3 weeks of age, portions of the PRRSV-infected ($n = 29$) and noninfected ($n = 29$) pigs were vaccinated with one of three commercially available vaccines, as described in Table 1. At 5 weeks of age, 2 groups ($n = 20$) were each given a booster dose of vaccines. At 10 weeks of age, 79 pigs were challenged with PCV2b. All pigs were necropsied at 13 weeks of age. Blood samples were collected from each group on the day of arrival at the facility and weekly thereafter until necropsy. The serum samples collected on the day of PCV2 challenge and at days postchallenge (dpc) 7, 14, and 21 were tested for the presence and amount of PRRSV RNA by reverse

transcription (RT) real-time PCR and for PCV2 DNA by quantitative real-time PCR. The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC; approval number 8-08-6618-S).

PRRSV inoculation. At 2 weeks of age, 51 pigs (in the PRRSV-Fort Dodge Animal Health (FDAH)-PCV2, PRRSV-Boehringer Ingelheim Vetmedica Inc. (BIVI)-PCV2, PRRSV-Intervet-PCV2, PRRSV, and PRRSV-PCV2 groups) were inoculated with approximately 10^5 50% tissue culture infective doses ($TCID_{50}$) of PRRSV isolate VR2385 as described previously (40). Each pig received 2 ml of the PRRSV inoculum intranasally by slowly dripping 1 ml into each nostril.

Vaccination. Three commercial vaccines were used in this experimental study. At 3 weeks of age, pigs in the PRRSV-FDAH-PCV2 and FDAH-PCV2 groups were vaccinated intramuscularly with 2 ml of Suvaxyn PCV2 vaccine (serial number 1861220A; Fort Dodge Animal Health, Inc., Fort Dodge, IA). Similarly, pigs in the PRRSV-BIVI-PCV2 and BIVI-PCV2 groups were vaccinated intramuscularly with 1 ml of Ingelvac CircoFLEX vaccine (serial number 309-136; Boehringer Ingelheim Vetmedica Inc.) at 3 weeks of age. Pigs in the PRRSV-Intervet-PCV2 and Intervet-PCV2 groups were vaccinated intramuscularly using 2 ml of Circumvent vaccine (serial number 02137920; Intervet Schering-Plough Animal Health) at 3 and 5 weeks of age.

PCV2 challenge. At 10 weeks of age, pigs in groups PRRSV-FDAH-PCV2, PRRSV-BIVI-PCV2, PRRSV-Intervet-PCV2, FDAH-PCV2, BIVI-PCV2, Intervet-PCV2, PRRSV-PCV2, and PCV2 were challenged with PCV2b. PCV2b isolate NC16845 (31) was used at a dose of $10^{4.5}$ $TCID_{50}$ per ml. Each pig received 2 ml intranasally and 2 ml intramuscularly.

Clinical evaluation. All the pigs were weighed on the day of arrival, the day of vaccination (3 and 5 weeks of age), the day of challenge (10 weeks of age; 0 dpc), and the day of necropsy (21 dpc); and the average daily weight gain (ADWG) was calculated. Other clinical parameters were not evaluated.

Anti-PCV2 IgG antibodies. All serum samples collected prior to PCV2 challenge (2, 3, 4, 5, 6, 7, 8, 9, and 10 weeks of age) and at dpc 7, 14, and 21 were tested using a SERELISA PCV2 Ab Mono blocking kit (Synbiotics Europe, Lyon, France) according to the manufacturer's instructions. Results were expressed as a sample-to-negative corrected (SNc) ratio. Samples were considered positive for the presence of anti-PCV2 antibodies if the calculated SNc ratio was less than or equal to 0.50, and they were considered negative if the SNc ratio was greater than 0.50.

PCV2 DNA detection and quantification. The DNA from serum samples collected at dpc 7, 14, and 21 was extracted using a QIAamp DNA minikit (Qiagen, Valencia, CA). The DNA was tested for the presence and amount of PCV2 DNA using a quantitative real-time PCR as described previously (33).

Anti-PRRSV IgG antibodies. Serum samples collected from pigs at 2 weeks of age and at dpc 0 and 21 were tested for the presence of antibodies to PRRSV (HerdChek X3 PRRS virus antibody test kit; IDEXX Laboratories Inc., Westbrook, MA). A sample-to-positive (S/P) ratio of equal to or greater than 0.4 was considered a positive result.

PRRSV RNA detection and quantification. RNA extraction from serum samples collected at the day of PCV2 vaccination, 7 days after PCV2 vaccination, and dpc, 0, 7, 14, and 21 from all the PRRSV-inoculated groups was performed using a QIAamp viral RNA minikit (Qiagen). Detection and quantification of PRRSV genomic RNA from the RNA extracts was achieved by real-time RT-PCR as described previously (40).

Necropsy. All pigs were humanely euthanized by pentobarbital overdose (Fatal-plus; Vortex Pharmaceutical, Ltd; Dearborn, MI) and necropsied at 13 weeks of age (21 dpc). The total amount of lung surfaces affected by macroscopic lesions (ranging from 0 to 100%) (14) and size of lymph nodes, with scores ranging from 0 (normal size) to 3 (four times the normal size) (32), were estimated in a blinded fashion. Sections of liver, spleen, colon, kidney, lymph nodes (mesenteric, superficial inguinal, tracheobronchial, and mediastinal), ileum, tonsil, and thymus were collected at necropsy; fixed in 10% neutral-buffered formalin; and processed routinely for histological examination.

Histopathology. Microscopic lesions were evaluated by a veterinary pathologist (T.O.) blinded to the treatment groups. Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and were scored from 0 (none) to 3 (severe). Lung sections were scored for the presence and severity of interstitial pneumonia, with scores ranging from 0 (normal) to 6 (severe, diffuse) (14). 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), and histiocytic inflammation and replacements of follicles, with scores ranging from 0 (normal) to 3 (severe) (32).

IHC. Immunohistochemistry (IHC) staining for the detection of PCV2 antigen was conducted on selected formalin-fixed and paraffin-embedded sections of

TABLE 2. ADWG from 0 to 21 dpc

Group	ADWG $\pm$ SE (kg)[a]
PRRSV-FDAH-PCV2	1.02 ± 0.03[A,B,C]
FDAH-PCV2	0.96 ± 0.03[B,C]
PRRSV-BIVI-PCV2	1.12 ± 0.04[A,B]
BIVI-PCV2	1.03 ± 0.03[A,B,C]
PRRSV-Intervet-PCV2	0.99 ± 0.05[B,C]
Intervet-PCV2	1.00 ± 0.05[A,B,C]
PRRSV	0.98 ± 0.06[B,C]
PCV2	0.91 ± 0.05[C]
PRRSV-PCV2	0.98 ± 0.06[B,C]
Negative controls	1.21 ± 0.06[A]

[a] Different superscripts (A, B, and C) within the same column represent significant differences ($P < 0.05$) between groups.

lymph nodes (mesenteric, superficial inguinal, tracheobronchial, and mediastinal), spleen, tonsil, and thymus using a rabbit polyclonal antiserum (42). PCV2 antigen scoring was done by a pathologist blinded to the treatment status of the animals, with scores ranging from 0 (no signal) to 3 (abundant amount of PCV2 antigen) as described previously (32).

Overall lymphoid lesion score. The overall microscopic lymphoid lesion score, which accounts for lymphoid depletion, histiocytic inflammation, and amount of PCV2 antigen, was calculated as previously described (32).

Statistical analysis. Statistical analysis of the data was performed using JMP software, version 8.0.1 (SAS Institute, Cary, NC). Summary statistics were calculated for all groups to assess the overall quality of the data, including normality. Continuous data were analyzed using the one-way analysis of variance (ANOVA). A P value of less than 0.05 was set as a statistically significant level throughout this study. If an ANOVA was significant, pairwise testing using Tukey's adjustment was performed to assess specific group differences. Real-time PCR results (number of copies per ml of serum) were $\log_{10}$ transformed prior to statistical analysis. Percent reduction for PCR data was measured as follows: $100 - [(100 \times \text{mean} \log_{10} \text{ number of genomic copies/ml in the vaccinated group})/(\text{mean} \log_{10} \text{ number of genomic copies/ml in positive-control animals})]$. Nonrepeated measures of necropsy and histopathology data were assessed using a nonparametric Kruskal-Wallis one-way ANOVA. If this nonparametric ANOVA test was significant ($P < 0.05$), then pairwise Wilcoxon tests were used to assess differences between groups. In order to determine differences in prevalence, a chi-square test was used.

RESULTS

ADWG. There were no significant ($P > 0.05$) differences in initial body weights among the groups of pigs at arrival in the research facility. After PRRSV infection, between 2 and 3 weeks of age, the ADWG in PRRSV-infected pigs was significantly ($P < 0.05$) lower than that in noninfected pigs (0.25 ± 0.01 kg versus 0.28 ± 0.01 kg). Similarly, from 3 to 10 weeks of age, the ADWG in PRRSV-infected pigs was significantly ($P < 0.0001$) lower than that in noninfected pigs (0.66 ± 0.01 kg versus 0.77 ± 0.01 kg). PCV2 vaccination had no effect on ADGW, as there was no significant ($P > 0.05$) difference between the vaccinated and nonvaccinated groups from 3 to 10 weeks of age. The ADWG for the individual groups from the time of PCV2 challenge until necropsy (10 to 13 weeks of age) is summarized in Table 2. There was no significant ($P > 0.05$) difference between PRRSV-infected and noninfected pigs or between vaccinated and nonvaccinated pigs. However, the ADWG in PCV2-infected pigs was significantly ($P < 0.05$) lower than that in noninfected pigs (1.00 ± 0.02 kg versus 1.08 ± 0.05 kg).

Anti-PCV2 IgG antibody levels. At arrival, 95/99 pigs had passively acquired PCV2 antibodies, with group mean SNc ratios between 0.00 and 0.10 (Fig. 1), and no significant differ-

ences were observed among the different treatment groups. The group mean PCV2 ELISA SNc ratios in vaccinated pigs were significantly ($P < 0.0001$) lower than those in nonvaccinated pigs between 5 weeks of age (vaccinated pigs, 0.22 ± 0.04; nonvaccinated pigs, 0.39 ± 0.04) and 10 weeks of age (vaccinated pigs, 0.06 ± 0.02; nonvaccinated pigs, 0.51 ± 0.04). Significantly ($P < 0.05$) higher SNc ratios (lower levels of antibody) were observed in the BIVI groups than in the FDAH and Intervet groups during the growing period; however, this was independent of PRRSV status. At the day of PCV2 challenge at 10 weeks of age, 98% (58/59) of the vaccinated pigs had detectable anti-PCV2 antibodies, whereas only 42.5% (17/40) of the nonvaccinated pigs were seropositive. After PCV2 challenge (dpc 7, 14, and 21), vaccinated groups had significantly ($P < 0.001$) lower SNc ratios than nonvaccinated challenge pigs, which were independent of the product used or PRRSV infection status at the time of vaccination and at 14 and 21 dpc.

Prevalence and amount of PCV2 DNA in serum. Pigs in the negative-control group and in the PRRSV group remained negative for PCV2 DNA throughout the study period. After PCV2 challenge, PCV2 DNA was detected in the majority of the nonvaccinated pigs at 7 (70%), 14 (100%), and 21 (100%) dpc. Among the vaccinated pigs, PCV2 viremia was limited to a few individual pigs (Table 3). Vaccination significantly ($P < 0.05$) reduced the amount of PCV2 DNA compared to that in nonvaccinated pigs (Table 3). The mean reductions of PCV2 viremia in the serum at 21 dpc were 88.6% for FDAH-PCV2, 88.8% for BIVI-PCV2, 90.2% for Intervet-PCV2, 89.4% for PRRSV-FDAH-PCV2, 66.8% for PRRSV-BIVI-PCV2, and 100% for PRRSV-Intervet-PCV2.

Anti-PRRSV IgG antibody levels. All pigs were negative for anti-PRRSV antibodies at the start of the study at 2 weeks of age (data not shown). All pigs that were not experimentally infected with PRRSV remained seronegative for PRRSV until the termination of the study at 13 weeks of age. At 10 weeks of age (dpc 0), the mean PRRSV ELISA S/P ratio values were 2.30 ± 0.11 for PRRSV-infected, non-PCV2-vaccinated pigs ($n = 21$) and 2.17 ± 0.09 for PRRSV-infected, PCV2-vaccinated pigs ($n = 30$), with no significant ($P > 0.05$) difference
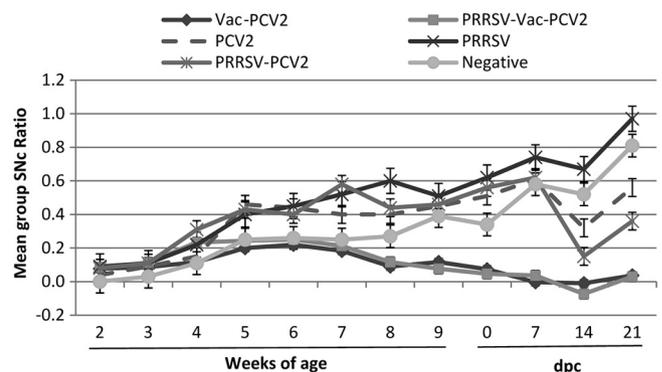

FIG. 1. Mean anti-PCV2 IgG antibody responses in the different treatment groups following PCV2 challenge. PRRSV inoculation, the first vaccination (Vac), challenge, and necropsy were performed at 2, 3, 10, and 13 weeks of age, respectively. Samples with an SNc ratio of 0.5 or greater are considered negative. Error bars indicate standard errors.

TABLE 3. PCV2 DNA-positive pigs among all pigs in each group at different days after PCV2 challenge

Group	No. of pigs PCV2 DNA positive/no. of pigs in group on the following days after PCV2 challenge[a]:		
	7	14	21
PRRSV-FDAH-PCV2	1/10 (0.42 ± 0.4)[A]	0/10 (0.00 ± 0.0)[A]	0/10 (0.00 ± 0.0)[A]
FDAH-PCV2	1/10 (0.38 ± 0.4)[A]	0/10 (0.00 ± 0.0)[A]	0/10 (0.00 ± 0.0)[A]
PRRSV-BIVI-PCV2	2/10 (0.89 ± 0.6)[A]	3/10 (1.56 ± 0.8)[B]	2/10 (0.81 ± 0.5)[A]
BIVI-PCV2	4/9 (1.98 ± 0.8)[AB]	2/9 (0.92 ± 0.6)[BA]	1/9 (0.41 ± 0.4)[A]
PRRSV-Intervet-PCV2	0/10 (0.00 ± 0.0)[A]	0/10 (0.00 ± 0.0)[A]	0/10 (0.00 ± 0.0)[A]
Intervet-PCV2	0/10 (0.00 ± 0.0)[A]	0/10 (0.00 ± 0.0)[A]	1/10 (0.36 ± 0.4)[A]
PRRSV	0/11 (0.00 ± 0.0)[A]	0/11 (0.00 ± 0.0)[A]	0/11 (0.00 ± 0.0)[A]
PCV2	7/10 (3.67 ± 0.8)[B]	10/10 (6.69 ± 0.4)[C]	10/10 (5.96 ± 0.3)[B]
PRRSV-PCV2	7/10 (3.94 ± 0.9)[B]	10/10 (7.22 ± 0.2)[C]	10/10 (7.23 ± 0.3)[B]
Negative controls	0/9 (0.00 ± 0.0)[A]	0/9 (0.00 ± 0.0)[A,B]	0/9 (0.00 ± 0.0)[A]

[a] Different superscripts (A, B, and C) within the same column represent significant differences ($P < 0.05$) in the mean amount of $\log_{10}$ PCV2 DNA between groups. Values in parentheses represent the mean $\log_{10}$ number of PCV2 DNA copies ± standard error for the group.

existing between groups. Similarly, there was no significant ($P > 0.05$) difference in mean group PRRSV S/P ratios on dpc 21 between PCV2-vaccinated (1.78 ± 0.11) and nonvaccinated (2.08 ± 0.13) pigs or between PCV2-challenged (1.87 ± 0.10) and nonchallenged (2.04 ± 0.17) pigs.

Prevalence and amount of PRRSV RNA in serum. At the time of PCV2 vaccination (7 days after PRRSV inoculation) and 7 days after PCV2 vaccination (14 days after PRRSV inoculation), 100% of the pigs in the PRRSV-challenged groups were positive for PRRSV RNA in serum, with PRRSV RNA loads ranging from 6.16 to 6.66 $\log_{10}$ copies/ml (Table 4). From 0 to 21 dpc, low levels of PRRSV RNA were detected in a few (1 to 3) pigs in all PRRSV-infected groups (Table 4). No significant ($P > 0.05$) differences were observed for PRRSV prevalence or RNA loads among the different treatment groups.

Macroscopic and microscopic lesions and PCV2 antigen in lesions. Macroscopic lung lesions were characterized by failure of the lungs to collapse and by focal-to-diffuse, mottled tan areas of pneumonia. Vaccinated, PCV2-challenged pigs ($n = 59$) had significantly ($P < 0.05$) lower mean lung scores than nonvaccinated PCV2-infected pigs ($n = 20$) (1.97 ± 0.32 versus 3.95 ± 1.00). Moreover, PRRSV-negative PCV2-challenged pigs ($n = 39$) had significantly ($P < 0.05$) lower mean lung scores than PRRSV-positive PCV2-challenged pigs ($n = 40$) (1.46 ± 0.28 versus 3.45 ± 0.62). There was no difference in the severity of macroscopic lung lesions among vaccinated groups ($P > 0.05$). In the PRRSV-PCV2 group, 9/10 pigs had lymph nodes of 2 to 3 times the normal size. In the remaining groups,

individual pigs (1 to 2 per group) had mildly enlarged lymph nodes.

Microscopic lesions were characterized by mild to severe lymphoid depletion and mild to moderate histiocytic replacement in lymphoid tissues. Among vaccinated pigs, 83.3% (49/59) had normal lymphoid tissues and 16.9% (10/59) had mild lymphoid lesions (overall lymphoid score, 1 or 2). Nonvaccinated and PCV2-infected pigs however, had the following lesion distribution: 10% (2/20) had normal lymphoid tissues, 45% (9/20) had mild lymphoid lesions (score, 1, 2, or 3), 40% (8/20) had moderate lymphoid lesions (score, 4, 5, or 6), and 5% (1/20) had severe PCV2-associated lesions (score, 7). There was no difference in lesion severity between PRRSV-infected and noninfected pigs.

PCV2 antigen was detected in 8/10 pigs in the PRRSV-PCV2 group, 7/10 pigs in the PCV2 group, and 0/59 pigs in the vaccinated groups; the differences were statistically significant ($P < 0.05$).

DISCUSSION

Various commercially available killed PCV2 vaccines are being used worldwide, and their efficacies have been demonstrated under both experimental conditions (13, 25, 29, 30, 40) and field conditions (5, 12, 17, 19, 39). PCV2 vaccination is recommended for usage in weaned pigs at about 3 weeks of age or older. However, at this age many pigs derived from PRRSV-positive breeding herds are infected with PRRSV, which is thought to negatively impact PCV2 vaccine efficacy. In the

TABLE 4. PRRSV RNA-positive pigs among all pigs in each group for pigs experimentally inoculated with PRRSV 7 days prior to PCV2 vaccination and 8 weeks prior to PCV2 challenge

Group	No. of pigs PRRSV RNA positive/no. of pigs in group on the indicated days after[a]:					
	PCV2 vaccination		PCV2 challenge			
	0	7	0	7	14	21
PRRSV-FDAH-PCV2	10/10 (6.16 ± 0.3)	10/10 (6.66 ± 0.3)	0/10 (0.00 ± 0.0)	1/10 (0.43 ± 0.4)	2/10 (0.96 ± 0.6)	0/10 (0.00 ± 0.0)
PRRSV-BIVI-PCV2	10/10 (6.35 ± 0.1)	10/10 (6.40 ± 0.3)	0/10 (0.00 ± 0.0)	1/10 (0.32 ± 0.3)	2/10 (0.66 ± 0.4)	2/10 (0.67 ± 0.5)
PRRSV-Intervet-PCV2	10/10 (6.29 ± 0.2)	10/10 (6.33 ± 0.2)	2/10 (0.63 ± 0.4)	1/10 (0.34 ± 0.3)	3/10 (1.07 ± 0.6)	2/10 (0.00 ± 0.0)
PRRSV	11/11 (6.48 ± 0.2)	11/11 (6.59 ± 0.4)	0/11 (0.00 ± 0.0)	1/11 (0.32 ± 0.3)	1/11 (0.37 ± 0.4)	0/11 (0.00 ± 0.0)
PRRSV-PCV2	10/10 (6.44 ± 0.3)	10/10 (6.29 ± 0.4)	2/10 (0.69 ± 0.5)	1/10 (0.28 ± 0.3)	2/10 (0.83 ± 0.6)	2/10 (0.79 ± 0.5)

[a] Values in parentheses represent the mean $\log_{10}$ PRRSV RNA copies ± standard error for the group.

present study, we evaluated PCV2 vaccine efficacy in PRRSV-positive conventional pigs by experimental inoculation with a known virulent isolate of PRRSV (14, 24, 28). It is well-known that PRRSV strains differ in virulence (28), and the results of this study may be different with other PRRSV challenge isolates.

Increased PCV2 replication and clinical PCVAD were observed in pigs experimentally coinfected with PRRSV and PCV2 (2, 16, 38). Although PRRSV-infected pigs had significantly increased macroscopic lung lesions in the present study, significant differences in the severity of microscopic lesions or amount of PCV2 antigen were not observed in PCV2-challenged pigs. A possible explanation for the discrepancy in observations is the difference in timing of pathogen administration: in the current study, the pigs were infected with PRRSV at 2 weeks of age, followed by PCV2 challenge at 10 weeks of age, whereas previously, coinfections were conducted at the same time (16) or within an interval of a few days (2, 38) and were done in younger pigs (1 to 35 days of age).

Although PCV2 vaccines are in general very effective, cases of apparent vaccine failures continue to occur. Vaccine failures under field conditions may be due to many reasons, including failure to follow the proper administration protocols and to use the recommended dose of the vaccines (30). The field strains might be different from the vaccine strains, and the breed of the pigs can also influence the outcome of PCVAD (22, 30).

The presence of high levels of passively derived anti-PCV2 antibodies at the time of vaccination has also been associated with decreased vaccine efficacy and vaccine failures. Experimental proof of this is lacking to date, as available research results indicate no adverse effect of maternally derived immunity on PCV2 vaccine efficacy (13, 29, 40). Similarly, the results of the current study indicate that PCV2 vaccination in the face of high levels of passively derived anti-PCV2 immunity was effective in inducing a humoral immune response. At 10 weeks of age, a significant higher prevalence of seropositive pigs was found in the vaccinated group than in the nonvaccinated control group.

Several studies have investigated the effect of PRRSV infection on vaccine efficacy but provided conflicting results. PRRSV infection at the time of classical swine fever virus vaccination significantly inhibited the host immune response and resulted in vaccination failure after subsequent exposure to classical swine fever virus (7, 43). For pseudorabies virus vaccination, although PRRSV infection affected the duration of the T-lymphocyte response, it did not inhibit the efficacy of the vaccine, which was capable of inducing protective immunity (7). In the present study, our data revealed that all the commercial vaccines used in this study were able to induce a protective immunity against PCV2 in the face of PRRSV infection at the time of vaccination. The difference in the experimental results might be due to different timings of infection with PRRSV, vaccination, and challenge. In the present study, pigs were challenged 5 weeks (two dose administrations) to 7 weeks (one dose administration) after vaccination, in contrast to a study where challenge occurred 3 weeks after initial vaccination (43).

In a previous study (26), we found that PCV2 infection had adverse effects on the efficacy of a modified live virus (MLV) PRRSV vaccine. The different effects of PRRSV and PCV2 infection on the efficacy of vaccines may be due to the different mechanisms that these two viruses use to induce immunosuppression (replication in macrophages versus lymphoid depletion), which are still not completely understood, and the types of vaccine (attenuated live versus inactivated) tested.

All PCV2 vaccines used, regardless of PRRSV infection status at the time of vaccination, were able to suppress PCV2 replication after PCV2 challenge, as demonstrated by significantly reduced levels of PCV2 viremia and significantly reduced amounts of PCV2 antigen in lymphoid tissues. The results of our study demonstrated that by 21 dpc there was no difference in the prevalence of PCV2 DNA in vaccinated groups regardless of PRRSV infection status at the time of vaccination or the vaccine product used. We conclude that prior PRRSV infection does not have an adverse effect on commercial PCV2 vaccination in conventional growing pigs and that pigs can be effectively immunized against PCV2 in the face of maternal antibodies.

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