Comparison of Two Commercial Type 1 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Modified Live Vaccines against Heterologous Type 1 and Type 2 PRRSV Challenge in Growing Pigs

Taeyeon Kim, Changhoon Park, Kyuhyung Choi, Jiwoo Jeong, Ikjae Kang, Su-Jin Park, Chanhee Chae
Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, Gwanak-gu, Seoul, Republic of Korea

The objective of the present study was to compare the efficacy of two commercial type 1 porcine reproductive and respiratory syndrome virus (PRRSV) modified live vaccines against heterologous type 1 and type 2 PRRSV challenge in growing pigs. Vaccination with a type 1 PRRSV vaccine reduced the level of viremia after type 1 PRRSV challenge but did not reduce the level of viremia after the type 2 PRRSV challenge in pigs. Increased levels of interleukin-10 (IL-10) stimulated by type 2 PRRSV coincided with the low numbers of type 2 PRRSV-specific interferon gamma-secreting cells (IFN-γ-SC) in vaccinated pigs after type 2 PRRSV challenge, whereas low levels of IL-10 stimulated by type 1 PRRSV coincided with high numbers of type 1 PRRSV-specific IFN-γ-SC in vaccinated pigs after type 1 PRRSV challenge. Additionally, vaccination with the type 1 PRRSV vaccine effectively reduced the lung lesions and type 1 PRRSV nucleic acids in type 1 PRRSV-challenged pigs but did not reduce lung lesions and type 2 PRRSV nucleic acids in type 2 PRRSV-challenged pigs. There were no significant differences between two commercial type 1 PRRSV vaccines against type 1 and type 2 PRRSV challenge based on virological results, immunological responses, and pathological outcomes. This study demonstrates that vaccinating pigs with the type 1 PRRSV vaccine provides partial protection against respiratory disease with heterologous type 1 PRRSV challenge but no protection with heterologous type 2 PRRSV challenge.

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is one of the most economically devastating diseases facing pig production worldwide. The infection of growing pigs with PRRSV causes severe respiratory diseases, leading to impaired growth in postweaned and growing pigs (1). The virus can also give rise to reproductive failure in sows (1). PRRSV is a small, positive-sense, enveloped, single-stranded RNA virus belonging to the family Arteriviridae in the order Nidovirales (2). PRRSV is classified into type 1 (European) and type 2 (North American) genotypes based on the 3′-terminal structural genes or the entire genome (3, 4). Type 1 PRRSV is further divided into three subtypes: a pan-European subtype 1 and East European subtypes 2 and 3, with nucleocapsid protein sizes of 128, 125, and 124 amino acids, respectively (5).

In South Korea, type 2 PRRSV was first isolated in 1994 (6). Until 2005, only type 2 PRRSV was detected in Korean swine herds (7, 8). The Korean type 1 isolates belonged to the pan-European subtype 1 (9, 10). Since then, both type 1 and type 2 PRRSVs have been circulating in the field. Infection with type 2 PRRSV only (54.4% [37/68 swine herds]) is the most prevalent, followed by infection with type 1 PRRSV only (29.4% [20/68 swine herds]) and co-infection with type 1 and type 2 PRRSV (16.2% [11/68 swine herds]) (10). The type 2 PRRSV modified live vaccine (Ingelvac PRRS modified live vaccine [MLV]; Boehringer Ingelheim) was first introduced to control PRRS in South Korea in 1996. Recently, two commercial type 1 PRRSV modified live vaccines (Porcilis PRRS [MSD Animal Health, Summit, NJ], and Unistain PRRS [Hipra, Amer, Spain]) were first licensed in 2014 in South Korea for the control of type 1 PRRS infection.

Since the first introduction of type 1 PRRSV vaccines, cross-protection is a major clinical issue because of the coexistence of the type 1 and type 2 PRRSVs in swine herds. Previous cross-protection studies have provided inconsistent results (11, 12). The Porcilis PRRS vaccine (MSD Animal Health) provides no protection against heterologous type 2 PRRSV challenge (11), whereas the Amervac PRRS vaccine (Hipra) provides partial protection against heterologous type 2 PRRSV challenge (12). However, a comparison of two commercial type 1 PRRSV-based modified live vaccines against type 1 and type 2 PRRSV challenge has yet to be undertaken. Hence, the objective of this study was to compare the efficacy of two commercial type 1 PRRSV-based modified live vaccines against heterologous type 1 and type 2 PRRSV challenge in growing pigs based on clinical, immunological, virological, and pathological outcomes.

MATERIALS AND METHODS

**PRRSV inocula.** Type 1 (strain SNUVR090485, pan-European subtype 1, GenBank accession no. JN315686) and type 2 (strain SNUVR090851, lineage 1, GenBank accession no. JN315685) PRRSVs were used as inocula. The type 1 SNUVR090485 virus was isolated from lung samples from an aborted fetus in southwestern Gyeonggi Province in 2009 (13). The type 2
SNUVR090851 virus was isolated from lung samples from different newly weaned pigs in Chungcheong Province in 2010 (14).

Experimental design. A total of 112 colostrum-fed, cross-bred, conventional piglets were purchased at 14 days of age from a commercial PRRSV-free farm. All piglets were negative for PRRSV, porcine circovirus type 2, swine influenza virus, and Mycoplasma hypopneumoniae, according to routine serological testing. All piglets were negative for type 1 and type 2 PRRSV viremia by real-time PCR.

All piglets were moved to a research facility, housed individually in separate rooms, and randomly allocated into 7 groups (n = 16 per group) using the random number generation function in Excel (Microsoft Corporation, Redmond, WA) (Table 1). Two commercial type 1 PRRSV vaccines (VacA [Porcilis PRRS] and VacB [Unistrain PRRS]) were used and administered according to the manufacturer’s instructions with regard to timing (28 days of age), dose (2.0 ml), and route of injection (intramuscularly in the right side of the neck).

At 63 days of age (0 days postchallenge [dpc]), the pigs in the VacA/challenge 1 (Ch1), VacB/Ch1, and unvaccinated (UnVac)/Ch1 groups were inoculated intranasally with 3 ml of tissue culture fluid containing 10⁵ 50% tissue culture infective doses (TCID₅₀)/ml of type 1 PRRSV (SNUVR090485, second passage in alveolar macrophages). The pigs in the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups were inoculated intranasally with 3 ml of tissue culture fluid containing 10⁵ TCID₅₀/ml of type 2 PRRSV (SNUVR090851 strain, second passage in MARC-145 cells). The inoculations were performed by setting the pigs on their buttocks perpendicularly to the floor and extending their necks fully back. The inoculum was slowly dripped into both nostrils of the pigs, taking approximately 3 to 5 min/pig. The UnVac/unchallenged (UnCh) pigs served as the negative-control group.

Blood samples and nasal swabs were collected at 35, 32, 30, 28, 25, 21, 14, 0, 3, 7, 10, and 14 dpc. Four pigs from each group were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 3, 7, 10 and 14 dpc, as previously described (15). Tissue samples were collected from each pig at necropsy. All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use Committee.

Clinical observation. Following vaccination and PRRSV challenge, the pigs were monitored weekly for their physical condition and scored daily for their clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (16). Observers were blinded to vaccination status. Rectal temperatures were recorded daily at the same time each day by the same personnel.

Quantification of PRRSV RNA in blood. RNA was extracted from serum samples and nasal swabs to quantify PRRSV genomic cDNA copy numbers, as previously described (17). Real-time PCR for the two vaccine strains and two challenge strains was designed to detect open reading frame 5 (ORF5) of the two vaccine strains and ORF7 of the two challenge strains, as determined by BioEdit software version 7.0.0 (T. Hall, North Carolina State University, Raleigh, NC).

For the Porcilis vaccine virus, the forward and reverse primers were 5'-TGTAGACACCGGGGGAG-3' and 5'-CTAGGCCTCCCATTTG CTCAG-3', respectively. For the Unistrain vaccine virus, the forward and reverse primers were 5'-GGTGGCCAGGCCATTGTGAC-3' and 5'-CACG CTGCTGAGTACATTCC-3', respectively. For the challenge strain type 1 PRRSV, the forward and reverse primers were 5'-GGTGGCCAGGCCATT TTGAC-3' and 5'-CACGCTGCTGAGTACATTCC-3', respectively. For the challenge strain type 2 PRRSV, the forward and reverse primers were 5'-GGCCAGCTGCTAATCAAAC-3' and 5'-AATGGATTGCAAGCG AGGGAA-3', respectively.

A standard curve for each set of primers was obtained. The PCR products for the two vaccine viruses (Porcilis vaccine virus, 166 bp; Unistrain vaccine virus, 169 bp) and the two challenge viruses (type 1 PRRSV, 184 bp; type 2 virus, 187 bp) were cloned with the TOPOCloner Blunt kit (Invitrogen, Carlsbad, CA) and propagated in Escherichia coli strain DH5α competent cells (Enzymtics), according to the manufacturer’s instructions. Plasmid DNA was purified with a plasmid purification kit (iNtRON Biotechnology, Seongnam, Gyeonggi, South Korea) and quantified using a spectrophotometer.

Amplification was carried out in a 20-µl reaction mixture containing 10 µl of Maxima SYBR green/ROX quantitative PCR (qPCR) master mix (Thermo Scientific, Billerica, MA), 7 µl of diethyl pyrocarbonate (DEPC) distilled water (DW), and 2 µl of cDNA. The thermal profile for SYBR green PCR was 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Each standard curve was generated using serially diluted plasmid standards of 2.6 × 10² to 2.6 × 10⁹ copies/µl (Porcilis vaccine virus plasmid), 4.4 × 10² to 4.4 × 10⁹ copies/µl (Unistrain vaccine virus plasmid), 6.3 × 10² to 6.3 × 10⁹ copies/µl (challenge type 1 PRRSV plasmid), and 8.5 × 10² to 8.5 × 10⁹ copies/µl (type 2 challenge PRRSV plasmid). In each run, positive and negative reference samples were tested along with the unknown samples. The melting curves of each amplification product were analyzed to verify the specificity of the PCR. The coefficients of variation for the cycle threshold (C_T) value obtained for each dilution were calculated. A sample was considered positive if the cycle threshold level was obtained at ≤40 cycles (18).

Serology. Serum samples were tested using a commercially available PRRSV enzyme-linked immunosorbent assay (ELISA) (HerdCheck PRRS X3 antibody [Ab] test; Idexx Laboratories, Westbrook, ME), according to the manufacturer’s instructions. Serum virus neutralization (SVN) tests were also performed using homologous (vaccine strain) and heterologous (challenge virus) viruses (19). The presence of virus-specific cytopathic effect (CPE) in each well was recorded after incubation for 7 days. Serum samples were considered to be positive for neutralizing antibodies (NAb) if the titer was >2.0 (log₂).

Enzyme-linked immunosorbent assay. The number of PRRSV-specific interferon gamma-secreting cells (IFN-γ-SC) using the challenge virus was determined in peripheral blood mononuclear cells (PBMCs), as previously described (20, 21). An assessment of PRRSV-specific IFN-γ-SC was determined by an enzyme-linked immunosorbtent (ELISPOT) assay (Mabtech, Mariemont, OH), according to the manufacturer’s instructions. In brief, 5 × 10⁵ PBMCs were plated on a 96-well microplate precoated with 10 µg/ml swine specific IFN-γ antibody (Mabtech). The cells were incubated overnight in either type 1 or type 2 challenge PRRSV (multiplicity of infection [MOI], 0.1). The spots on the membranes were read by an automated ELISPOT reader (AID EliSpot Reader; AID GmbH, Strasburg, Germany). The results were expressed as the number of IFN-γ-SC per 1 million PBMCs.

Quantification of interleukin-10 secretion. The levels of interleukin-10 (IL-10) were quantified in the supernatants of PBMC (2 × 10⁵ cells per well, 250 µl) cultures in vitro for 20 h with the type 1 challenge PRRSV (MOI, 0.01) or phytohemagglutinin (PHA) (10 µg/ml) by using commercial ELISA kits (swine interleukin-10 ELISA kit; Invitrogen, Camarillo, CA), according to the manufacturer’s instructions. The detection limit for IL-10 was 3.0 pg/ml.

In situ hybridization. The PCR products for the type 1 and type 2 PRRSVs were generated from the type 1 and type 2 challenge PRRSVs by PCR (18). The PCR products were purified with Wizard PCR prep kits (Promega, Madison, WI). The purified PCR product was labeled by random

### TABLE 1 Study design with vaccination and challenge status

| Group       | Vaccination with | PRRSV challenge with |
|-------------|------------------|----------------------|
| VacA/Ch1    | Porcilis PRRS    | 1                    |
| VacA/Ch2    | Porcilis PRRS    | 2                    |
| VacB/Ch1    | Unistrain PRRS   | 1                    |
| VacB/Ch2    | Unistrain PRRS   | 2                    |
| UnVac/Ch1   |                  | 1                    |
| UnVac/Ch2   |                  | 2                    |
| UnVac/UnCh  |                  |                      |

*There were 16 animals in each group, and necropsy was performed at 3, 7, 10, and 14 dpc in all cases.
priming with digoxigenin-dUTP using a commercial kit (Boehringer Mannheim, Indianapolis, IN). After fixation, tissues from each pig were dehydrated through a graded series of alcohol solutions and a xylene step and embedded in paraffin wax. Sections (4 μm) were prepared from each tissue and then processed for in situ hybridization to detect in situ viral RNA. In situ hybridization for the detection of type 1 and type 2 PRRSV nucleic acids in lung tissues was performed and analyzed morphometrically, as previously described (14).

Morphometric analysis. Macroscopic and microscopic lung lesions were observed and scored as previously described (16). Two pathologists scoring the lung sections on a scale ranging from 0 (normal) to 4 (severe diffuse) were blind to the treatment of the animal from which the pulmonary tissue samples being examined were obtained.

Statistical analysis. Prior to statistical analysis, all real-time PCR and NAb data were transformed to log_{10} and log_{2} values, respectively. The normality of the distribution of the examined variables was evaluated by the Shapiro-Wilk test. Continuous data (rectal temperature, PRRSV RNA, PRRSV-specific IFN-γ-SC, IL-10, PRRSV antigen score, and macroscopic lung lesion score) were analyzed using a one-way analysis of variance (ANOVA). If the ANOVA showed a significant effect, Tukey’s test for multiple comparisons was performed at each time point. Discrete data (microscopic lung lesion scores, respiratory scores, and the proportion of pigs with viremia) were analyzed by Mann-Whitney tests. A P value of <0.05 was considered significant.

RESULTS

Clinical observation. The mean rectal temperatures were significantly higher (P < 0.05) in pigs from the VacA/Ch1, VacB/Ch2, and UnVac/Ch1 groups than in pigs from the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups at 3 to 13 dpc. The health of the pigs from the VacA/Ch1, VacB/Ch1, and UnVac/UnCh groups remained normal throughout the study, as measured by their respiratory scores and rectal temperatures (Fig. 2).

Quantification of PRRSV RNA in blood. No genomic copies of type 1 and type 2 PRRSV were detected in the serum of any pig at the time of vaccination (−35 dpc). Genomic copies of the vaccine strain were detected in the sera of vaccinated challenged pigs (VacA/Ch1, VacA/Ch2, VacB/Ch1, and VacB/Ch2 groups) at −21 dpc (14 days postvaccination). Thereafter, no vaccine strain was detected in the sera of the vaccinated challenged pigs.

The prevalence rates of type 1 and type 2 PRRSV are summarized in Table 2. Pigs from the VacA/Ch1 and VacB/Ch1 groups had significantly lower (P < 0.05) percentages of viremic animals than did pigs from the UnVac/Ch1 group at 3, 7, and 10 dpc. The percentages of viremic pigs were not significantly different among 3 groups (VacA/Ch2, VacB/Ch2, and UnVac/Ch2) throughout the experiment.

Genomic copies of type 1 PRRSV were detected in the sera of pigs from the VacA/Ch1, VacB/Ch1, and UnVac/Ch1 groups at 3, 7, 10, and 14 dpc. Pigs from the VacA/Ch1 and VacB/Ch1 groups had significantly fewer (P < 0.05) genomic copies of type 1 PRRSV in their sera at 3, 7, 10, and 14 dpc than did pigs from the UnVac/Ch1 group. However, there were no significant differences between the VacA/Ch1 and VacB/Ch1 groups in terms of their genomic copy numbers of type 1 PRRSV throughout the experiment (Fig. 3).

Genomic copies of type 2 PRRSV were detected in the sera of pigs from the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups at 3, 7, 10, and 14 dpc. However, there were no significant differences
among 3 groups (VacA/Ch2, VacB/Ch2, and UnVac/Ch2) (Fig. 3). No PRRSV of any genotype was detected in the sera of pigs from the UnVac/UnCh group throughout the experiment.

**Anti-PRRSV antibodies.** At the time of PRRSV vaccination (4 weeks of age; 35 dpc), pigs in all 7 groups were seronegative. Antibodies specific for PRRSV were detected by ELISA in vaccinated challenged pigs (VacA/Ch1, VacA/Ch2, VacB/Ch1, and VacB/Ch2 groups) from 25 dpc (10 days postvaccination) onward and in unvaccinated challenged pigs (UnVac/Ch1 and UnVac/Ch2 groups) from 10 dpc onward. The anti-PRRSV antibody titers were significantly higher \((P < 0.05)\) in vaccinated challenged pigs (VacA/Ch1, VacA/Ch2, VacB/Ch1, and VacB/Ch2 groups) than in unvaccinated challenged (UnVac/Ch1 and UnVac/Ch2 groups) pigs at 3, 7, 10, and 14 dpc. Anti-PRRSV antibody titers were not detected in pigs from the UnVac/UnCh group at any time.

**PRRSV-specific neutralizing antibodies.** Homologous (against the vaccine strain) and heterologous (against the challenge virus) NAb titers were not detected in any pigs from any group (NAb titer, <2 log2) throughout the experiment.

**PRRSV-specific interferon-γ-secreting cells.** When PBMCs were stimulated with type 1 challenge PRRSV, the numbers of type 1 PRRSV-specific IFN-γ-SC of pigs from VacA/Ch1 and VacB/Ch1 began to increase, reached an average (± standard deviation) of 28.4 ± 13 cells/10^6 PBMCs at −21 dpc, and returned to basal levels (<20 cells/10^6 PBMCs) at −14 dpc. Upon challenge with type 1 PRRSV, pigs from the VacA/Ch1 and VacB/Ch1 groups had significantly higher \((P < 0.05)\) numbers of type 1 PRRSV-specific IFN-γ-SC than pigs from the UnVac/Ch1 group at 0, 3, 7, 10, and 14 dpc (Fig. 4A). No type 1 PRRSV-specific IFN-γ-SC was detected in pigs from the UnVac/UnCh group throughout the experiment.

When PBMCs were stimulated with the type 2 challenge PRRSV, the mean frequencies of type 2 PRRSV-specific IFN-γ-SC remained at basal levels (<20 cells/10^6 PBMCs) in pigs from the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups until −7 dpc. Upon challenge with type 2 PRRSV, pigs from the VacA/Ch2 and VacB/Ch2 groups had significantly higher \((P < 0.05)\) numbers of type 2 PRRSV-specific IFN-γ-SC than pigs from the UnVac/Ch2 group at 0, 3, 7, 10, and 14 dpc. The numbers of type 2 PRRSV-specific IFN-γ-SC in pigs from the VacA/Ch2 and VacB/Ch2 groups were significantly lower \((P < 0.05)\) at 3 dpc than at 0 dpc (Fig. 4B). No type 2 PRRSV-specific IFN-γ-SC was detected in pigs from the UnVac/UnCh group throughout the experiment.

When equivalent series of IFN-γ-ELISPOT results were compared between type 1 PRRSV stimulation versus type 2 PRRSV stimulation within the same vaccinated groups, stimulation with type 1 PRRSV produced significantly higher \((P < 0.05)\) numbers of IFN-γ-SC at 3, 7, 10, and 14 dpc than stimulation with type 2 PRRSV.

**Interleukin-10.** After stimulation with type 1 PRRSV, IL-10 was not detected in pigs from the UnVac/Ch1 group until 0 dpc. Upon challenge with type 1 PRRSV, IL-10 reached maximal levels at 3 and 7 dpc and thereafter decreased gradually until 14 dpc in the UnVac/Ch1 group. High levels of IL-10 were detected at 3 and 7 dpc only in pigs from the VacA/Ch1 and VacB/Ch1 groups (Fig. 5A). IL-10 was not detected in pigs from the UnVac/UnCh group throughout the experiment.

After stimulation with type 2 PRRSV, IL-10 was not detected in pigs from the UnVac/Ch2 group until 0 dpc. Upon challenge with type 2 PRRSV, IL-10 reached maximal levels at 3 dpc and thereafter decreased gradually until 14 dpc in the UnVac/Ch2 group. High levels of IL-10 were detected at 3 dpc only in pigs from the VacA/Ch2 and VacB/Ch2 groups (Fig. 5B). IL-10 was not detected in pigs from the UnVac/UnCh group throughout the experiment.

When equivalent series of IL-10 results were compared between type 1 PRRSV stimulation versus type 2 PRRSV stimulation...
within the same vaccinated groups, stimulation with type 2 PRRSV produced significantly higher (P < 0.05) levels of IL-10 at 3 dpc than did stimulation with type 1 PRRSV.

**Macroscopic and microscopic lung lesions.** Pigs from the VacA/Ch1 and VacB/Ch1 groups had significantly lower (P < 0.05) scores for macroscopic and microscopic lung lesions than pigs in the UnVac/Ch1 group at 7, 10, and 14 dpc (Table 2).

**In situ hybridization.** Type 1 PRRSV-positive cells were detected only in pigs from the VacA/Ch1, VacB/Ch1, and UnVac/Ch1 groups, while type 2 PRRSV-positive cells were detected only in pigs from the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups (Fig. 6). Pigs in the VacA/Ch1 and VacB/Ch1 groups had significantly lower (P < 0.05) scores for the mean number of type 1 PRRSV-positive cells per unit area of lung than pigs in the UnVac/Ch1 group at 7, 10, and 14 dpc (Table 2). Type 1 PRRSV-positive cells were not detected in the lungs of pigs from the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups. Type 2 PRRSV-positive cells were not detected in the lungs of pigs from the VacA/Ch2, VacB/Ch2, and UnVac/Ch2 groups.

**DISCUSSION**

The results of the present study demonstrate that the two type 1 PRRSV vaccines provide partial protection against respiratory disease caused by heterologous type 1 PRRSV challenge but no protection against heterologous type 2 PRRSV challenge in pigs during the acute phase. In addition, no significant differences between two type 1 PRRSV vaccines against type 1 and type 2 PRRSV challenge viruses were found in this study based on virological results, immunological responses, and pathological outcomes.

Our results agree with previous findings in which vaccination with the type 1 PRRSV-based vaccine (Porcilis PRRS) provides partial protection against pan-European subtype 1 and East European subtype 3 PRRSVs (20, 21). In addition, the type 1 PRRSV-based vaccine (Porcilis PRRS) has a lack of cross-protection against type 2 PRRSV (11). These cross-protection results are in contrast with another study in which vaccination with type 1 PRRSV (Amervac PRRS; Hipra) provided only partial protection against challenge with heterologous type 2 PRRSV (12). However,

**TABLE 2 Results of lesion scoring and numbers of PRRSV-positive cells within lung lesions on different days postchallenge**

| Group      | dpc<sup>a</sup> | 1 infected/total no. with type:<br>Prevalence of PRRSV viremia | Lung lesion score (mean ± standard deviation)<br>Microscopic | PRRSV-positive cells (mean ± standard deviation) within lung lesion of type:<br>Microscopic |
|------------|------------------|-------------------------------------------------|------------------|------------------|
| VacA/Ch1   | 3                | 2/16                                            | 13.7 ± 4.7       | 0.75 ± 0.95      |
|            | 7                | 3/12                                            | 12.5 ± 8.6       | 3.35 ± 3.21      |
|            | 10               | 0/8                                             | 17.5 ± 9.5       | 2.6 ± 2.05       |
|            | 14               | 0/4                                             | 7.5 ± 2.8        | 0.5 ± 1          |
|            | 15/16            |                                                 | 2.22 ± 0.4       | 0                |
|            | 14               | 0/4                                             | 65 ± 12.9        | 3.02 ± 0.45      |
|            | 4/4              |                                                 | 0.44 ± 0.38      | 0                |
| VacA/Ch2   | 3                | 0/16                                            | 34 ± 4.5         | 14 ± 5.47        |
|            | 7                | 0/12                                            | 86.2 ± 4.7       | 44.5 ± 5.68      |
|            | 10               | 0/8                                             | 86.2 ± 7.5       | 45.07 ± 6.41     |
|            | 14               | 0/4                                             | 65 ± 12.9        | 35.27 ± 4.11     |
|            | 14               | 0/4                                             | 8.7 ± 2.5        | 0.75 ± 1.5       |
| VacB/Ch1   | 3                | 2/16                                            | 17.5 ± 9.5       | 7.5 ± 0.95       |
|            | 7                | 2/12                                            | 16.2 ± 11        | 2.75 ± 2.06      |
|            | 10               | 0/8                                             | 11.2 ± 6.2       | 2.75 ± 0.5       |
|            | 14               | 0/4                                             | 8.7 ± 2.5        | 0.75 ± 1.5       |
| VacB/Ch2   | 3                | 0/16                                            | 38.7 ± 15.4      | 12.75 ± 3.59     |
|            | 7                | 0/12                                            | 87.5 ± 5         | 49.5 ± 5.06      |
|            | 10               | 0/8                                             | 83.7 ± 4.7       | 40 ± 9.27        |
|            | 14               | 0/4                                             | 60 ± 8.1         | 31 ± 9.83        |
| UnVac/Ch1  | 3                | 10/16                                           | 20 ± 7           | 3.6 ± 2.38       |
|            | 7                | 12/12                                           | 33.7 ± 7.5       | 20.25 ± 3.3      |
|            | 10               | 8/8                                             | 37.5 ± 9.5       | 18.57 ± 6.03     |
|            | 14               | 3/4                                             | 15 ± 10.8        | 8.6 ± 1.91       |
| UnVac/Ch2  | 3                | 0/16                                            | 37.5 ± 9.5       | 11.17 ± 5.77     |
|            | 7                | 0/12                                            | 85 ± 10          | 51.5 ± 4.93      |
|            | 10               | 8/8                                             | 80 ± 8.1         | 43.25 ± 8.22     |
|            | 14               | 0/4                                             | 67.5 ± 9.5       | 40.75 ± 8.01     |
| UnVac/UnCh | 3                | 0/16                                            | 0.2 ± 0.1        | 0                |
|            | 7                | 0/12                                            | 0.2 ± 0.2        | 0                |
|            | 10               | 0/8                                             | 0.4 ± 0.5        | 0                |
|            | 14               | 0/4                                             | 0.3 ± 0.2        | 0                |

<sup>a</sup> dpc, days postchallenge.

<sup>b</sup> Different letters (c to f) indicate significant (P < 0.05) differences among groups.
there are no statistically significant differences in the lung lesion scores and levels of viremia between vaccinated and unvaccinated animals, despite the fact that vaccinated animals show improvement in respiratory clinical signs (12). Therefore, the cross-protection by type 1 PRRSV-based modified live vaccine against type 2 PRRSV is limited, because pathological outcomes and virological (viremia) results are critical parameters for the evaluation of vaccine efficacy. In addition, discrepant cross-protection results between previous (12) and present studies may be due to genetic diversity, because the nucleotide sequence homology of ORF5 between two type 1 challenge viruses is 86.8%.

The selection of a proper challenge virus is critical when comparing two type 1 PRRSV-based modified live vaccines. The type 1 challenge virus was isolated from pigs in 2009 and is not a vaccine virus. The two commercial type 1 PRRSV-based modified live vaccines were introduced in South Korea in 2014. The type 1 challenge virus is a pan-European subtype 1 virus, as are most field viruses in South Korea (7, 8). The type 1 challenge virus that was chosen was not closely related to either vaccine virus and had similar levels of homology to the two vaccine viruses based on their ORF5 nucleotide sequences. Strain SNUVR090485 has homologies of 87.9% and 88.1% to the Porcilis and Unistrain vaccine viruses, respectively. In addition, the type 2 challenge virus (lineage 1) is not a vaccine virus.

**FIG 3** Mean values of the genomic copy number of type 1 (A) and type 2 (B) PRRSV RNA in the serum of pigs from VacA/Ch1 (■), VacA/Ch2 (□), VacB/Ch1 (▲), VacB/Ch2 (△), UnVac/Ch1 (●), and UnVac/Ch2 (○) groups. Different letters (a, b, and c) indicate significant (P < 0.05) differences between groups. Error bars indicate standard deviations.
virus, as the commercial type 2 PRRSV-based live vaccine is lineage 5 for Ingelvac PRRS MLV (Boehringer Ingelheim) and lineage 8 for Fostera PRRS (Zoetis) (22).

The viral loads in blood and pathological lesions are used to assess vaccine efficacy (11,12). The viral load is the actual viral nucleic acid quantity measured by real-time PCR. Vaccination with a type 1 PRRSV vaccine reduces the level of viremia after challenge with type 1 PRRSV but cannot reduce the level of viremia after challenge with the type 2 PRRSV in pigs, as reported previously (11,12). In the present study, the reduction in PRRSV viremia coincides with the appearance of PRRSV-specific IFN-γ-SC. Interestingly, the two type 1 PRRSV vaccines induce higher frequencies of type 1 PRRSV-specific IFN-γ-SC than type 2 PRRSV-specific IFN-γ-SC after challenge. These differences may result in the different levels of reduction of type 1 and type 2 PRRSV viremia. Nevertheless, in another study, the significance of IFN-γ-SC is not known, since there is no association with control of infection (23). Further studies are needed to determine the protective role of IFN-γ-SC in vaccinated challenged pigs.

PRRSV infection enhances systemic IL-10 production in infected pigs, especially during acute infection (24, 25). Since IL-10 is well known as a potent immunosuppressive cytokine (26), IL-10 production suppresses cell-mediated immune responses, particularly IFN-γ-SC responses, as previously reported (27). In the present study, increased levels of IL-10 stimulated by the type 2 PRRSV vaccine coincide with the low numbers of type 2 PRRSV-specific IFN-γ-SC.
IFN-γ-SC after type 2 PRRSV challenge, whereas low levels of IL-10 stimulated by the type 1 PRRSV vaccine coincide with the high numbers of type 1 PRRSV-specific IFN-γ-SC after type 1 PRRSV challenge. These differences in the induction of IL-10 and IFN-γ-SC by stimulation with vaccines with different genotypes of PRRSV may explain why the type 1 PRRSV vaccine led to different efficacies against type 1 and type 2 PRRSV challenges. In contrast, in a previous study, the role of IL-10 was controversial. Despite the fact that low levels of IL-10 coincide with high numbers of IFN-γ-SC in the present study, IL-10 levels in blood are not correlated with PRRSV infection status (28). Further studies are needed to determine whether this observation is a direct or indirect effect.

Pathological evaluation is also a critical parameter to evaluate vaccine efficacy because the most striking and consistent pathological lesions induced by PRRSV were interstitial pneumonia. The challenge strains of type 1 and type 2 PRRSV are virulent, resulting in the most extensive and severe interstitial pneumonia at 7 days postinfection, with lesions resolving between 14 and 21 days postinfection (13, 14). Based on previous pathological data, the type 1 PRRSV-based vaccine was evaluated in pigs at 3, 7, 10, and 14 dpc. Vaccination of pigs with the type 1 PRRSV vaccine significantly reduces lung lesions and type 1 PRRSV antigen scores within the lung lesions after type 1 PRRSV challenge, but it cannot reduce lung lesions and type 2 PRRSV antigen scores within the lung lesions after type 2 PRRSV challenge.

To our knowledge, this is the first experimental challenge study to evaluate and compare two commercial type 1 PRRSV-based...
modified live vaccines against heterologous type 1 and type 2 PRRSV challenge. The lack of cross-protection of the type 1 PRRS-based modified live vaccine against type 2 PRRSV challenge is clinically significant information because type 2 PRRSV induces more severe respiratory disease than type 1 PRRSV (14, 29, 30). Nonetheless, the antigenic diversity of the field strains of both genotypes is extreme, and the outcome of vaccination with type 1 PRRS-based modified live vaccines is difficult to predict. These results indicate that the control of certain PRRSV strains can be achieved more efficaciously by a same-genotype-based PRRSV vaccine due to the genetic and antigenic diversity of the field strains.

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