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Comparison of four commercial PRRSV MLV vaccines in herds with co-circulation of PRRSV-1 and PRRSV-2

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ABSTRACT

The efficacy of four commercial porcine reproductive and respiratory syndrome virus (PRRSV) modified-live virus (MLV) vaccines against respiratory disease was evaluated and compared in pig farms suffering from co-infection with PRRSV-1 and PRRSV-2. All vaccinated groups on average exhibited improved growth rate compared to the unvaccinated pigs. Interestingly, the two groups vaccinated with either of the PRRSV-2 MLV vaccines had a better overall growth rate compared to the pigs vaccinated with either of the PRRSV-1 MLV vaccines. Vaccination of pigs with either of the PRRSV-1 MLV vaccines did not result in reduction of PRRSV-1 or PRRSV-2 viremia whereas vaccination of pigs with either of the PRRSV-2 MLV vaccines resulted in the reduction of PRRSV-2 viremia only. Taken together, the results of this field study demonstrate that a PRRSV-2 MLV vaccine can be efficacious against respiratory disease caused by co-infection with PRRSV-1 and PRRSV-2.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically devastating diseases to the global swine industry. The causative agent for PRRS is the PRRS virus (PRRSV), which belongs to the newly reclassified genus *Porartevirus*, family *Arteriviridae*, and order *Nidovirales*. PRRSV has two distinct species: PRRSV-1 (European-like) and PRRSV-2 (North American-like) which are genetically, antigenically, and pathogenically distinct [1–3] and were recently reclassified as two separate species, PRRSV-1 and PRRSV-2 in the new taxonomy [4]. In Europe, PRRSV-1 is the prevailing virus with reproductive failure as the major clinical manifestation. In North America, PRRSV-2 is the predominant virus, and the symptoms include both reproductive failure in sows and respiratory disease in growing pigs.

In Korea, the current situation with PRRS is somewhat different compared to Europe and North America. PRRSV-2 is the more predominant virus, but both viruses are prevalent and cause reproductive failure in sows and respiratory disease in growing pigs. It is generally accepted that Korean PRRSV-2 induces a more severe respiratory disease compared to Korean PRRSV-1 [5] while both viruses have similar virulence in female reproductive failure [6]. A recent diagnostic analysis of cases submitted between January 2017 and June 2018 determined that out of 167 PRRSV positive serum samples collected from growing pigs, 46 were positive for PRRSV-1, 67 were positive for PRRSV-2, and 54 were positive for both PRRSV-1 and PRRSV-2. These cases underscore the need to control the respiratory disease caused by both PRRSV-1 and PRRSV-2 infection in growing pigs.

Currently, there are four modified-live virus (MLV) vaccines which are commercially available in Korea. Two are PRRSV-1 specific and the other two are PRRSV-2 specific. Only one of the PRRSV-1 MLV vaccines (www.hipra.com) and one of the PRRSV-2 MLV vaccines (www.boehringer-ingelheim.com) claims cross-protection against both species. The other PRRSV-2 MLV vaccine (www.zoetis.com) claims protection of pigs against PRRSV-2 but can cross-protect against PRRSV-1 under experimental conditions [7]. To date, no comparative evaluation of the efficacy of these vaccines has been performed under the same field conditions. The objective of the current study was to compare the efficacy of these four commercial PRRSV MLV vaccines in herds suffering from co-circulation of PRRSV-1 and PRRSV-2.

2. Material and methods

2.1. History of farm

The pig farm used for this study is a one-site 1000-sow herd with continuously farrowing units but with all-in-all-out nurseries and finishing barns. Pigs were vaccinated for *Mycoplasma hyopneumoniae* at 7

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days of age and porcine circovirus type 2 (PCV2) at 21 days of age. No clinical signs related to porcine circovirus associated disease were observed in any of the growing pigs. However, no PRRSV MLV vaccine was administered for at least one year. The farm experienced an epizootic of respiratory symptoms in 7–11 week-old growing pigs between September and November 2015. The morbidity was 20–25%, while the mortality in pigs with respiratory symptoms was 5–8%. Interestingly, no reproductive failure symptoms such as abortion, premature farrowing, stillborn, or weak-born piglets were observed in breeding females during this period. Four growing pigs with respiratory symptoms were submitted to the Department of Veterinary Pathology at the Seoul National University. At necropsy, diffuse grayish-yellow fibrinopurulent exudates overlied the pleural, pericardial, and peritoneal surface in 3 of the growing pigs at 88 days of age. Fresh lung samples from all four pigs were collected for virus isolation. Some lung samples were also fixed in 10% neutral buffered formalin for histopathology and *in situ* hybridization. PRRSV-1 (SNUVR150266, GenBank MG271757) was isolated in lung tissue from pig A. PRRSV-2 (SNUVR150267, GenBank MG385131) was isolated in lung tissue from pig B. A phylogenetic analysis was performed to compare the field isolates with the vaccine viruses based on the open reading frame 5 (ORF5) nucleotide sequence (Fig. 1). Histopathological lesions were characterized by typical interstitial pneumonia with increased numbers of interstitial and alveolar macrophages present. *In situ* hybridization indicated PRRSV-1 and PRRSV-2 infection of interstitial and alveolar macrophages.

2.2. Clinical field study design

The field study was performed in December 2015 according protocols that follow the guidelines of the Republic of Korea's Animal, Plant & Fisheries Quarantine & Inspection Agency (QIA, http://qia.go.kr). QIA guidelines require that 40 pigs are assigned to each vaccinated and control groups. Forty sows were selected and 5 piglets were collected from each sow (200 total piglets) with each of the five piglets from an individual sow randomly assigned to each of the five groups using the random number generation function (Excel, Microsoft Corporation, Redmond, WA, USA). To minimize sow variation, we selected piglets with minimal variation in weight and an equal number of male and female. At 28 days of age (0 days post vaccination, dpv), pigs from the Vac1A group were injected intramuscularly on the right side of the neck with 2 mL of Porcilis PRRS (MSD Animal Health, Lot No. D353 A07), pigs from the Vac1B group with UNISTRAIN PRRS (Hipra, Lot No. 61WK-B), pigs from the Vac2A group with Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica, Lot No. 245-659 A), and pigs from the Vac2B group with Fostera PRRS (Zoetis, Lot No. A405013B) according to each of the manufacturer's instructions. Pigs from the UnVac group were administered an equal volume of phosphate buffered saline (PBS, 0.01 M, pH 7.4) (Table 1). Upon vaccination, pigs from each of 5 groups were housed by treatment, with a minimum of four pens per treatment and 10 pigs per pen. Pens were randomly assigned to litters/treatments with an empty pen between each occupied pen to minimize the shedding of the vaccine virus to the individual pigs in the control group. All animals were housed within the same building in similar conditions, receiving the same feed and subjected to the same management practices. At 90 days of age (62 dpv), since they do not shed the virus anymore, pigs were allowed to commingle to minimize pen variation. They were randomly reassigned into 20 pens (10 pigs per pen) within the same building for the remainder of the study. The full length of the study was 140 days, from 28 (0 dpv) to 168 (140 dpv) days of age.

Blood samples from each piglet were collected by jugular venipuncture at 28 (0 dpv), 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. Necropsies were performed on piglets that had died of natural causes and lung tissue samples were collected for bacteria isolation (*Actinobacillus pleuropneumoniae*, *Haemophilus papasuis*, *Pasteurella multocida*, *Streptococcus suis*, and *Trueperella pyogenes*), histopathology, and *in situ* hybridization. RNA was extracted from lung homogenates. And ORF5 sequence was amplified from cDNA and sequenced as previously described [8,9]. All protocols were previously approved by the Seoul National University Institutional Animal Care and Use Committee.

2.3. Clinical observation

Clinical observation for respiratory symptoms was performed twice per week using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) [10]. Observers were blinded to vaccination and type of vaccine status. Mortality rate was calculated as the number of pigs that died divided by the number of pigs initially assigned to that group within batch. Pigs that died throughout the study were necropsied.

The live weight of each pig was measured at 28 (0 dpv), 49 (21 dpv), 70 (42 dpv), 112 (84 dpv), and 168 (140 dpv) days old. The average daily weight gain (ADWG; gram/pig/day) was analyzed over four time periods: (i) 28–49 days of age, (ii) 49–70 days of age, (iii) 70–112 days of age, and (iv) 112–168 days of age, ADWG during the different production stages was calculated as the difference between the starting and final weight divided by the duration of the stage. Calculation of the mean ADWG for each group was based only on the ADWG of the surviving pigs.

2.4. Sequencing of field viruses

Five serum samples among PCR positive samples from each group were randomly selected at 28 (0 dpv), 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. These serum samples were used for sequence analysis of ORF5 by polymerase chain reaction (PCR) amplification [8,9]. The PCR products were purified using a commercial kit (Wizard PCR Preps DNA Purification and PCR Clean-Up System, Promega, Madison, WI, USA), cloned with the TOPcloner Blunt kit (Enzynomics,

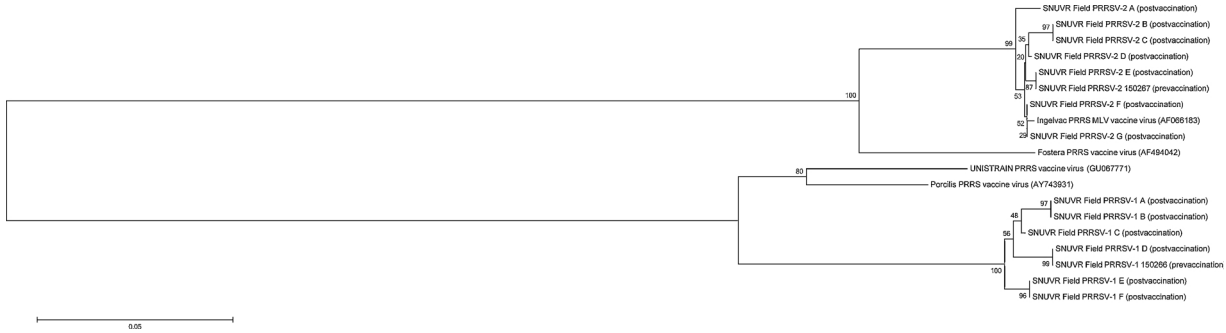

Fig. 1. Phylogenetic analysis. Open reading frame 5 genome from the field and the vaccine viruses. An unrooted neighbor-joining tree was constructed from aligned nucleotide sequences.

Table 1
Experimental design, and clinical and pathological results (mean ± standard error) among vaccinated and unvaccinated pigs under field conditions.

	Age (day)	Vac1A	Vac1B	Vac2A	Vac2B	UnVac
Vaccine		Porcilis PRRS	UNISTRAIN PRRS	Ingelvac PRRS MLV	Fostera PRRS	None
Vaccine type		PRRSV-1	PRRSV-1	PRRSV-2	PRRSV-2	None
No. of pigs		40	40	40	40	40
Mortality		2/40	2/40	2/40	1/20	4/40
Body weight (Kg)	28	6.32 ± 0.10	6.43 ± 0.08	6.41 ± 0.10	6.40 ± 0.10	6.38 ± 0.10
ADWG (gram/pig/day)	28-49	358.5 ± 358.6	358.6 ± 13.2	364.9 ± 14.8	372.0 ± 15.1	335.6 ± 15.8
	49-70	370.9 ± 384.7	384.7 ± 15.9†	473.7 ± 12.9*	479.9 ± 10.6*	281.7 ± 12.1‡
	70-112	706.1 ± 702.4	702.4 ± 13.9†	770.7 ± 17.5*	772.9 ± 14.9*	695.1 ± 17.0†
	112-168	746.7 ± 750.0	750.0 ± 13.7*	749.1 ± 12.4*	781.1 ± 13.1*	694.9 ± 10.0†
	28-168	619.9 ± 622.2	622.2 ± 5.1†	656.6 ± 5.3*	672.1 ± 6.7*	579.1 ± 5.6‡
Lung lesion score						
Macroscopic	168	32.9 ± 3.9†	32.6 ± 4.1†	24.2 ± 2.9†	23.3 ± 2.9†	50.8 ± 3.6*
Microscopic	168	1.34 ± 0.16†	1.34 ± 0.15†	1.05 ± 0.11†	1.05 ± 0.11†	2.14 ± 0.12*

Significant difference is indicated at P value < 0.05*,†,‡.

Daejeon, Korea), and propagated in DH5α competent cells (Enzynomics) according to the manufacturer's instructions. Plasmid DNA was purified with a plasmid purification kit (iNtRON Biotechnology, Sungnam, Kyeonggido, Korea) and sequenced by a commercial service (Sol Gent Co Ltd, Daejeon, Korea). Three clones of each PCR product were independently sequenced at least three times.

2.5. Quantification of PRRSV RNA

RNA was extracted from serum samples using a commercial kit (QIAamp Viral RNA Mini Kit, Qiagen, Seoul, Korea). Genomic cDNA copy numbers were quantified using real-time PCR [11]. Two different real-time PCRs were performed for the field and the vaccine viruses, respectively. For the detection of field strains, PRRSV-1 forward and reverse primers were 5'- TGGCCAGTCAGTCAATCAAC-3' and 5'-AATC GATTGCAA GCAGAGGGAA-3', respectively. PRRSV-2 forward and reverse primers were 5'-TGGCCAGTCAGTCAATCAAC-3' and 5'-AATCGA TTGCAAGCAGAGGGAA-3', respectively [11]. Real-time PCR for the quantification of genomic cDNA from the vaccine viruses, was performed as previously described [12–14].

2.6. Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc). Serum samples were considered positive for PRRSV antibody if the sample/positive (S/P) ratio was ≥0.4, according to the manufacturer's instructions.

2.7. Enzyme-linked immunospot assay

The numbers of PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) stimulated with the field viruses isolated from farm were determined in peripheral blood mononuclear cells (PBMC) as previously described [13,15,16]. PBMC seeded at (5×10^5 PBMCs per well) were stimulated with MARC-145 cell lysate (multiplicity of infection equivalent of 0.01) as the recall antigen for 20 h, incubating at 37 °C in a 5% CO_2 atmosphere. The IFN-γ positive spots on the membranes were imaged, analyzed and counted using an automated enzyme-linked immunospot (ELIPOT) assay ELISPOT Reader (AID ELISPOT Reader, AID GmbH, Strassberg, Germany). The results were expressed as the numbers of IFN-γ-SC per million PBMC. ELISPOT assay was repeated twice.

2.8. Pathology

The estimation of macroscopic lung lesions (ranging from 0 to 100% of the affected lung) was based on the percentage of the volume of the entire lung and the percentage volume from each lobe added to the entire lung score [10]. The total amount of microscopic lung lesions was scored blindly for each lung sections ranging from 0 (normal) to 4 (severe) by two independent pathologists and analyzed morphometrically with the NIH Image J 1.51 r Program (http://imagej.nih.gov/ij/download.html) [5,10].

2.9. Statistical analysis

The number of genomic copies of PRRSV data was log transformed prior to analysis. A generalized linear mixed model was used for all statistical comparisons with SAS version 9.3 (SAS Institute, Cary, NC) where group, time and their interaction were fixed effects while pigs were a random effect. A value of $P < 0.05$ was considered significant. The difference in mean response was assessed between groups.

3. Results

3.1. Clinical observation

The mean respiratory scores were significantly lower ($P < 0.05$) in pigs from all four vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) at 56 (28 dpv), 63 (35 dpv), 70 (42 dpv), and 84 (56 dpv) days old compared to the unvaccinated group (UnVac). Only pigs from the Vac1A and Vac1B groups had significantly lower ($P < 0.05$) mean respiratory scores compared to the UnVac group at 77 (49 dpv) days old (Fig. 2).

There was no significant difference in body weight among the Vac1A (average weight 6.32 kg ± 0.65), Vac1B (average weight 6.43 kg ± 0.51), Vac2A (average weight 6.41 kg ± 0.62), Vac2B (average weight 6.40 kg ± 0.61), and UnVacA (average weight 6.38 kg ± 0.61) groups at 28 days old. The ADWG was significantly higher ($P < 0.05$) in pigs from the Vac2A and Vac2B groups compared to pigs from the Vac1A, Vac1B, and UnVac groups during the 49–70 (21–42 dpv) and 70–112 (42–84 dpv) day period. The ADWG was significantly higher ($P < 0.05$) in pigs from the Vac1A, Vac1B, Vac2A, and Vac2B groups compared to pigs from the UnVac group during the 112–168 (84–140 dpv) day period. The ADWG was significantly higher ($P < 0.05$) in pigs from the Vac1A and Vac1B groups compared to pigs from the UnVac group during the 49–70 day period. The overall growth rate (28 to 168 days of age) of pigs from the Vac2A and Vac2B groups was significantly higher ($P < 0.05$) compared to pigs from the Vac1A, Vac1B, and UnVac groups. The overall growth rate (28 to 168 days of age) was significantly higher ($P < 0.05$) in pigs from the Vac1A and Vac1B groups compared to pigs from the UnVac group (Table 1).

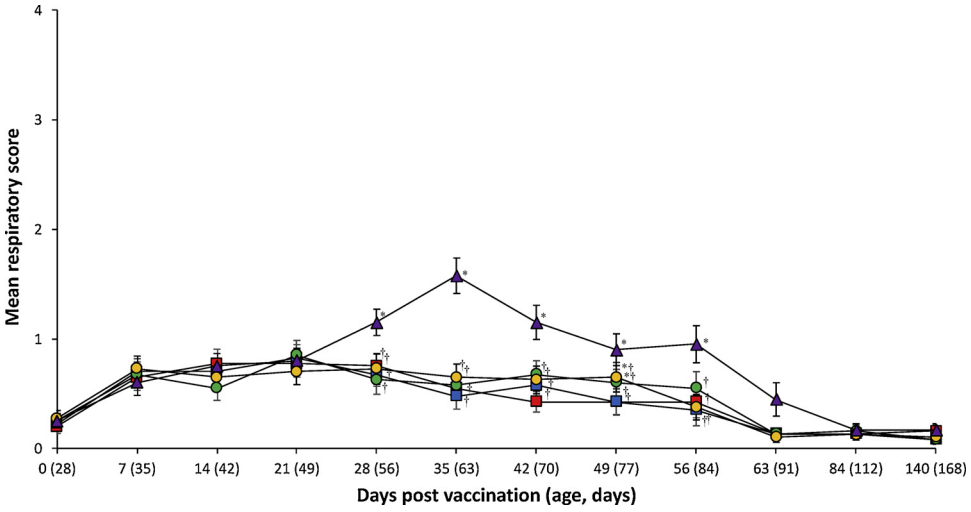

Fig. 2. Mean respiratory score in pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value $< 0.05^{*,\dagger}$.

3.2. Diagnosis of dead pigs

In the Vac1A group there were two pigs that died at 85 days old (57 dpv) and both exhibited symptoms of severe bronchointerstitial pneumonia. Both PRRSV-1 and PRRSV-2 were detected in interstitial and alveolar macrophages within the lung lesions by *in situ* hybridization. *P. multocida* was isolated in the pneumonic lung of one of the individual pigs. *H. parasuis* was isolated in diffuse grayish-yellow fibrinopurulent exudates overlining the pleural surface of the other individual pig. Vac1B group also had two pigs that died one at 85 (57 dpv) the other at 94 (66 dpv) days old. Both pigs had severe interstitial pneumonia with fibrinopurulent pleuritis. Interstitial and alveolar macrophages within lung lesions were positive for PRRSV-1 (Fig. 3A) and PRRSV-2 (Fig. 3B) as detected by *in situ* hybridization. *H. parasuis* was isolated in fibrinopurulent exudates overlining the pleural surface of both dead pigs. Two pigs from the Vac2A group died at 87 (59 dpv) and 88 (60 dpv) days old. The individual pig that died at 87 (59 dpv) days of age had severe interstitial pneumonia and *H. parasuis* was present in fibrinopurulent exudates overlining the pleural surface. The pig that died at 88 (60 dpv) days old had severe bronchointerstitial pneumonia and interstitial and alveolar macrophages within lung lesions were positive for PRRSV-1 and PRRSV-2 by *in situ* hybridization. *P. multocida* was also isolated from the pneumonic lung. The Vac2B group had only one pig that died at 94 (66 dpv) days old with severe interstitial pneumonia. Only PRRSV-2 could be detected in alveolar macrophages within lung lesions by *in situ* hybridization. *H. parasuis* was present in fibrinopurulent exudates overlining the pleural surface. The UnVac group had a total of four pigs die between 85 (57 dpv) to 99 (71 dpv) days of age. One pig died at 85 (57 dpv) days of age with severe pleuropneumonia and had PRRSV-1 positive macrophages within lung lesions. A second pig died at 86 (58 dpv) days of age with severe pleuropneumonia and had macrophages within lung lesions that were positive for both PRRSV-1 and PRRSV-2. Additionally, *A. pleuropneumoniae* was isolated from its pleuropneumonic lung. The last two pigs died at 96 (68 dpv) and 99 (71 dpv) days of age respectively and both had severe bronchointerstitial pneumonia. However, only the pig that died at 99 (71 dpv) days of age had PRRSV-2 positive interstitial and alveolar macrophages and had *H. parasuis* in fibrinopurulent exudates overlining the pleural surface.

3.3. Sequencing analysis

Lung samples from 11 total pigs that died during the field study from all 5 groups were collected and screened by PCR. Six of the samples were positive for PRRSV-1 and seven were positive for PRRSV-2. The PRRSV strains isolated from the farm prior to vaccination were

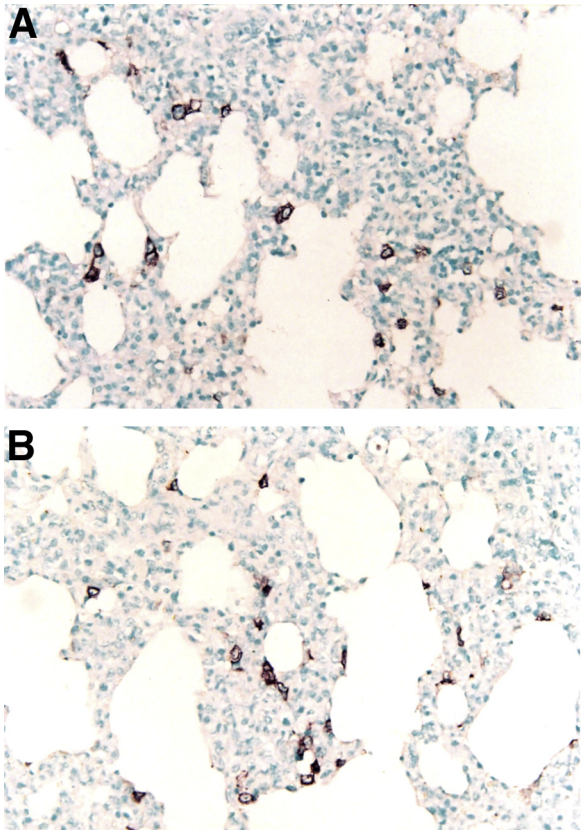

Fig. 3. *in situ* hybridization of PRRSV. PRRSV-1 nucleic acid was detected in interstitial macrophages in interstitial pneumonia (A). PRRSV-2 nucleic acid was detected in interstitial macrophages in interstitial pneumonia (B).

PRRSV-1 (SNUVR150266, GenBank MG271757) and PRRSV-2 (SNUVR150267, GenBank MG385131). A comparison of the ORF5 sequences of PRRSV-1 and PRRSV-2 isolates prior to vaccination with the vaccine strains, revealed only a 98.2–100% and 97.2–100% identity with PRRSV-1 (SNUVR150266, GenBank MG271757) and PRRSV-2 (SNUVR150267, GenBank MG385131) strains respectively (Fig. 1).

Sequence analysis of the 6 PRRSV-1 strains isolated in this field study revealed that they were highly homologous (97.8% to 100%) with the PRRSV-1 strain (SNUVR150266, GenBank MG271757), isolated from the same farm prior to PRRSV vaccination. Similarly, the 7 PRRSV-2 strains isolated during this study were highly homologous

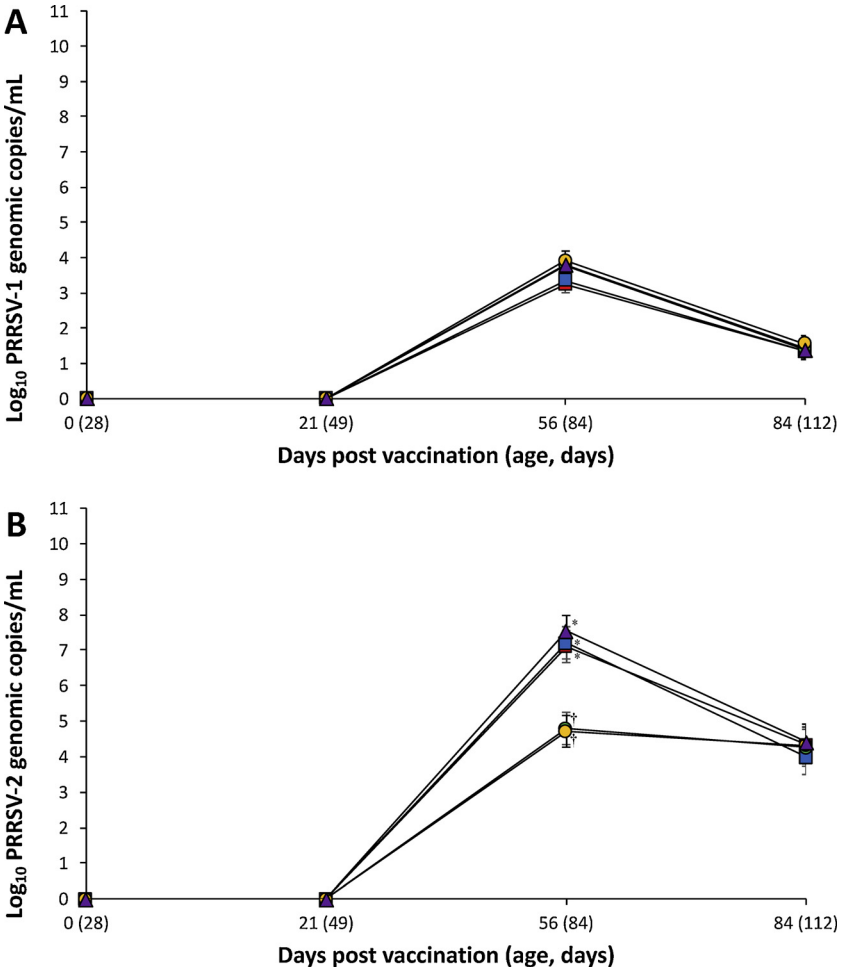

Fig. 4. Mean values of the genomic copy number of PRRSV-1 (A) and PRRSV-2 (B) PRRSV RNA in serum of pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value < 0.05 [*,†].

(98.8% to 100%) with the PRRSV-2 strain (SNUVR150267, GenBank MG385131) isolated from the same farm prior to PRRSV vaccination (Fig. 1).

The vaccine virus from the Vac1B group was detected in the serum sample of only one pig at 49 (21 dpv) days old. Vaccine virus from the Vac2A group was detected in serum samples at 49 (21 dpv, three pigs) and 84 (56 dpv, two pigs) days old. In Vac2B group, the vaccine virus was detected at 49 (21 dpv, one pig) and 84 (56 dpv, one pig) days old. Based on ORF5 sequencing following vacccination, no vaccine strain cross-contamination was observed among any of the vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B). None of the vaccine virus strains were detected in pigs from the UnVac group.

3.4. Quantification of PRRSV RNA in sera

No genomic copies of PRRSV were detected in the serum samples of any of the individual pigs at the time of vaccination (0 dpv, 28 days old). There were no significant differences in the number of genomic copies of PRRSV-1 RNA among the five groups throughout the study (Fig. 4A). However, pigs from the Vac2A and Vac2B groups had significantly lower ($P < 0.05$) number of genomic copies of PRRSV-2 RNA in their sera at 84 (56 dpv) days old compared to the Vac1A, Vac1B, and UnVac groups (Fig. 4B).

3.5. Serology

PRRSV ELISA was used to measure the presence of antibodies in serum samples. At the time of PRRSV vaccination (0 dpv, 28 days old), pigs in all five groups were seronegative for PRRSV. At 49 (21 dpv) and 84 (56 dpv) days old, pigs from all 4 vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) had significantly higher ($P < 0.05$) PRRSV antibodies compared to the unvaccinated group (UnVac). At 56 dpv, pigs from the Vac2A and Vac2B groups had significantly higher ($P < 0.05$) PRRSV antibodies compared to the Vac1A and Vac1B groups (Fig. 5).

3.6. Interferon-γ secreting cells

Pigs from all four of the vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) had a significantly ($P < 0.05$) higher numbers of PRRSV-1 and PRRSV-2 specific IFN-γ-SC in PBMC compared to the unvaccinated group (UnVac) at 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. At 84 (56 dpv) and 112 (84 dpv) days old, pigs from the Vac1A and Vac1B groups had a significantly higher ($P < 0.05$) numbers of PRRSV-1 specific IFN-γ-SC in PBMC compared to the Vac2A and Vac2B groups (Fig. 6A). Pigs from the Vac2A and Vac2B groups had a significantly higher ($P < 0.05$) number of PRRSV-2 specific IFN-γ-SC in PBMC compared to the Vac1A and Vac1B groups at 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. Lastly, pigs from the Vac2B group had significantly higher ($P < 0.05$) numbers of PRRSV-2 specific IFN-γ-SC in PBMC compared to pigs from the Vac2A group at 84 (56 dpv) days old (Fig. 6B).

3.7. Pathology

Pigs from all four vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) had significantly lower ($P < 0.05$) macroscopic and microscopic lung lesion scores compared to pigs from the unvaccinated group (UnVac) at 168 (140 dpv) days old. There were no significant

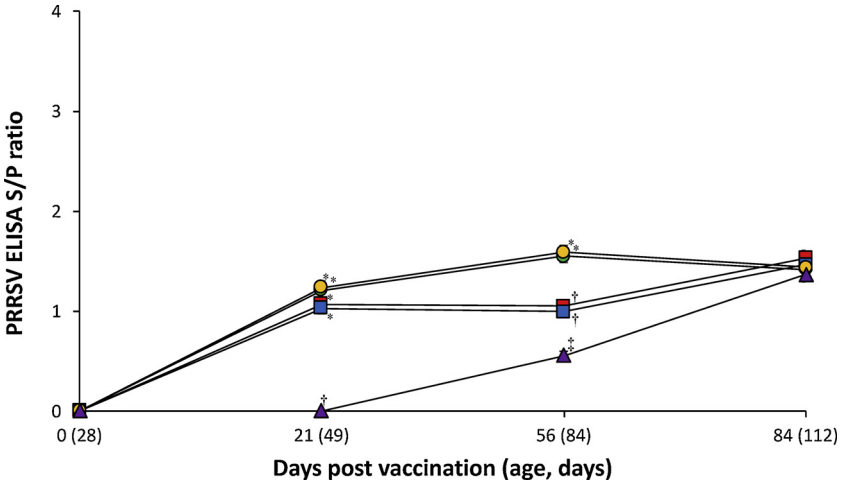

Fig. 5. Mean values of the PRRSV ELISA S/P ratio in serum of pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value $< 0.05^{*,\dagger,\ddagger}$.

differences among the vaccinated groups (Table 1).

4. Discussion

In this field study we evaluated the efficacy of four commercially available PRRSV MLV vaccines. Of the four vaccines, two were based on PRRSV-1 and two on PRRSV-2. The efficacies were evaluated under field conditions instead of a more controlled study because, they represent real life conditions where the vaccinated pigs are continuously exposed to field viruses circulating in the farm which can ultimately significantly affect the efficacy of a vaccine. Therefore, field trial results may not always agree with controlled studies but they are the ultimate "real world" data providing swine practitioners and producers with valuable data in selecting the right PRRSV vaccine for their farm. In this study, pigs were housed according to treatment groups until 88 days of age (60 days post vaccination) to avoid possible transmission of vaccine viruses between the different vaccinated groups within the same building. All conditions within the housing facility including air handling were the same for all groups ensuring that all individual pigs are exposed to similar field conditions at the same time. This field trial

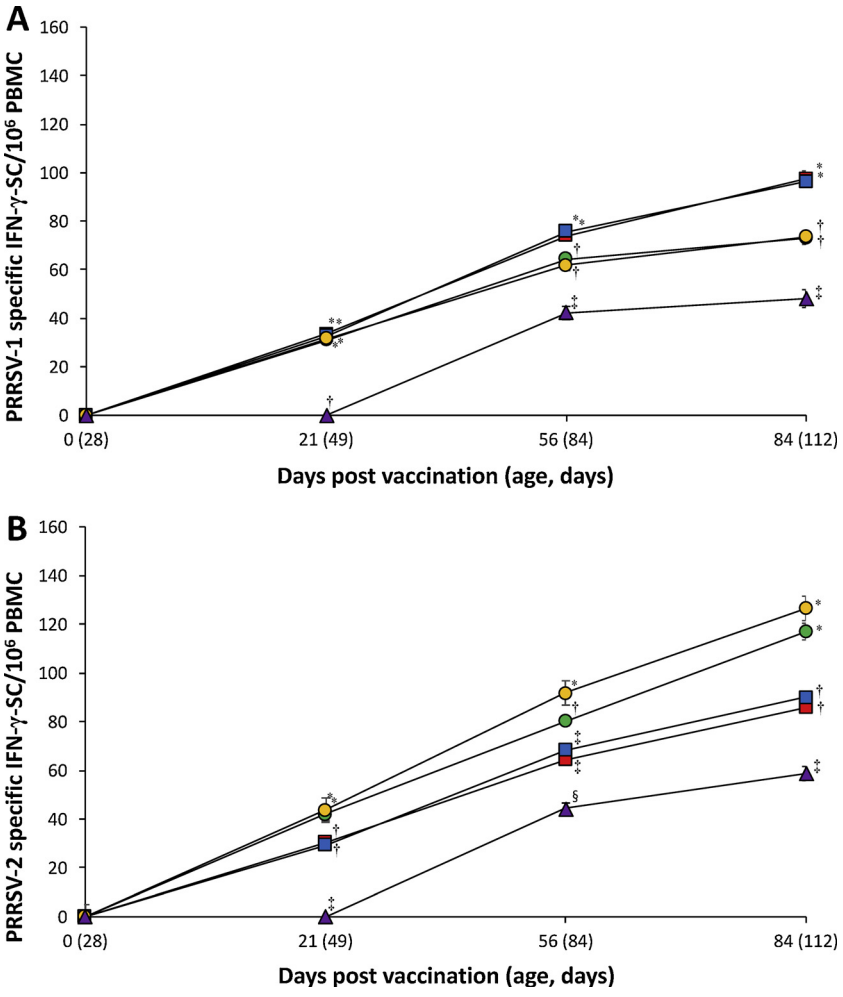

Fig. 6. Frequency of PRRSV-1 (A) and PRRSV-2 (B) specific IFN-γ-SC/10^6 PBMC in pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value $< 0.05^{*,\dagger,\ddagger,\S}$.

design combined with the broad sampling interval allows us to draw the conclusion that vaccination results in improved growth rate compared to unvaccinated groups.

Growth rate is one of the most important parameters in evaluating vaccine efficacy under field conditions because respiratory disease caused by PRRSV typically results in weight loss. Regardless of which vaccine or the vaccine type, vaccinated pigs showed better overall growth rate compared to unvaccinated pigs. There was also no significant difference in growth rate between each of the PRRSV-1 or PRRSV-2 based vaccines respectively. However, pigs vaccinated with PRRSV-2 MLV vaccines (both groups combined) exhibited a better growth rate compared to PRRSV-1 MLV vaccinated pigs (both groups combined). The differences in growth rate between PRRSV-1 MLV- and PRRSV-2 MLV-vaccinated groups may be due to genetic similarity between vaccine and field viruses. The identity between the field isolates and PRRSV-2 vaccine strains is 91.2–99.8% compared to 88.4–89.1% identity with the PRRSV-1 vaccine strains. There is also some evidence that the genetic similarity within field PRRSV strains may affect the efficacy of the same PRRSV MLV vaccine used in this study [17]. However, genetic similarity between vaccine virus and field virus does not always predict vaccine efficacy [18,19]. Further studies are needed to elucidate the relationship between vaccine efficacy and genetic similarities between vaccine and field strains. Another reason for the difference in growth rate observed between the two types of vaccines could be due to the difference in virulence between PRRSV-1 and PRRSV-2. In general, Korean PRRSV-2 is more virulent than Korean PRRSV-1 [5]. Therefore, in farms where both PRRSV types are circulating, protection against PRRSV-2 field strains by PRRSV-2 MLV vaccines can lead to a better growth rate compared to the protection against PRRSV-1 strains by the PRRSV-1- MLV vaccines.

Interestingly, even though vaccination with PRRSV-1 MLV vaccines did not result in reduction of PRRSV-1 viremia it did result in an improved the growth rate compared to unvaccinated pigs. Moreover, the same PRRSV-1 MLV vaccine used in this study also improved the growth rate and clinical signs in spite of not decreasing PRRSV-1 viremia in pig farms circulating PRRSV-1 only [20,21]. These results clearly suggest that PRRSV-1 infection can significantly hinder growth rate and that vaccination with PRRSV-1 MLV is highly beneficial.

Vaccination against PRRSV has vast economic benefits. The average market weight of PRRSV-1 MLV vaccinated pigs (both groups combined) increased by 5.87 Kg/pig compared to unvaccinated pigs (93.31 Kg in PRRSV-1 MLV vaccinated group *vs.* 87.44 Kg in unvaccinated group; $P < 0.05$). The improved market weight of 5.87 kg/pig increased revenue by approximately 13.80 US$ (exchange rate; US $1.00 = 1141 Korean Won) per pig. In PRRSV-2 MLV vaccinated pigs (both groups combined), the average market weight increased by 11.98 Kg/pig compared to unvaccinated pigs (99.42 Kg in PRRSV-2 MLV vaccinated group *vs.* 87.44 Kg in unvaccinated group; $P < 0.05$). The improved market weight of 11.98 kg/pig increased revenue by approximately 28.15 US$ (exchange rate; US $1.00 = 1141 Korean Won) per pig. Thus, this growth improvement had a clear economic impact on the pig farmers.

Another way that PRRSV can affect pigs is through an increased incidence of secondary bacterial infection, including *H. parasuis*, *P. multocida*, and *A. pleuropneumoniae* [22]. Infection of pigs with PRRSV-1 and PRRSV-2 followed by secondary bacterial infection can cause respiratory disease, leading to growth retardation and even death. In our study, several individual pigs that died, in addition to PRRSV infection were also infected with bacteria such as *P. multocida*, *H. parasuis*, and *A. pleuropneumoniae.* Vaccination of pigs in our study improved significantly the growth rate as well as the severity of respiratory disease, mortality rate and severity of lung lesions, compared to the unvaccinated group.

Cell-mediated immunity seems to play an important role in the protection against respiratory disease caused by PRRSV infection [23–25]. In a previous study with a controlled dual challenge, activation of T cell correlated with a reduction of PRRSV viremia [26,27]. In this field study, viral lysates of field PRRSV isolates from the same farm were used for the quantification of IFN-γ-SC. All four commercial vaccines were able to activate T cell responses against field viruses. As expected, PRRSV-1 MLV vaccines induced a stronger PRRSV-1 specific IFN-γ-SC response while PRRSV-2 MLV vaccines induced a stronger PRRSV-2 specific IFN-γ-SC response. Nonetheless, neither PRRSV-1 nor PRRSV-2 MLV vaccines were able to reduce PRRSV-1 viremia. In contrast, vaccination with either of the PRRSV-2 based MLV vaccines resulted in reduction of PRRSV-2 viremia. This suggests that induction of IFN-γ-SC does not always correlate with protection as reported in previous studies [28,29]. Alternatively, sequence variation between the four vaccine viruses and field PRRSV-1 isolates could definitely have an effect on immunodominance especially in PRRSV-T cell responses suggesting they may not be antigenically related. Further studies are needed to understand the difference in reduction of viremia between PRRSV-1 and PRRSV-2 based MLV vaccines.

We examined whether or not new viruses were introduced to the farm after vaccination. This is important because this could affect the efficacy of the PRRSV MLV vaccine. There were 11 pigs that died during this study between 85–96 days of age. In 5 out of the 11, both PRRSV-1 and PRRSV-2 were detected, indicating that both species were co-circulating in the population as early as 85 days of age (57 days post vaccination). Sequence analysis of ORF5 confirmed that field PRRSV-1 isolated post vaccination had a 97.8–100% identity with PRRSV-1 isolated prior to vaccination and PRRSV-2 isolated post vaccination had a 98.8–100% identity with PRRSV-2 isolated prior to vaccination. According to interpretation of the sequence analysis, 97–98% sequence identity indicates close relatedness of two viruses [30]. This suggests that no new PRRSV strains were introduced to the herd after vaccination.

Since transplacental infection is more than likely a main route of virus transmission in a herd it is important to note that PRRSV was not detected in the blood of 28-day-old pigs at the time of vaccination. All of the pigs in each of the vaccinated groups were exposed to the circulating viruses from the growing pig population, especially during the fattening period. Even though respiratory disease symptoms were recorded in vaccinated individuals, they did not correlate with the peak of the respiratory symptoms observed in unvaccinated animals around 63 days of age (35 days post vaccination). Those clinical signs could be attributed to other pathogens such as *M. hyopneumoniae* circulating in the population apart from PRRSV. After completion of our field study, the swine farmer did vaccinate with a mycoplasma vaccine at 21 days of age instead of 7 days of age and respiratory symptoms in pigs around 63 days of age were no longer observable.

In general, PRRSV-2 is more virulent and causes more severe respiratory disease in growing pigs compared to PRRSV-1 [5,31,32]. However, virulence and damage by the highly virulent PRRSV-1 Lena strain (subtype 3) in Europe is comparable to some Korean PRRSV-2 field strains [33]. In addition, Korean swine producers have recently observed unusual severe respiratory disease caused by highly virulent PRRSV-1 in their farms [34]. In this study the efficacy of the commercial vaccines was tested in a farm co-infected with regular virulence PRRSV-1 and PRRSV-2. Therefore, the conclusions drawn from the results in this study would be difficult to predict the level of efficacy in the case of a co-infection with a high virulent PRRSV-1 and typically virulent PRRSV-2. Our results however suggest that it is effective to use a PRRSV-2 MLV vaccine to prevent respiratory disease against co-infection with PRRSV-1 and PRRSV-2 under field conditions.

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