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A comparison of two commercially available porcine reproductive and respiratory syndrome virus (PRRSV) modified-live virus vaccines analyzing the growth performance in 1-day-old vaccinated swine located on endemic farms co-circulating PRRSV-1 and PRRSV-2

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ABSTRACT. The objective of this study was to compare the efficacy of a porcine reproductive and respiratory syndrome virus (PRRSV)-1 and PRRSV-2 modified-live virus (MLV) vaccines when administered at 1 day of age under field conditions. The piglets elicited anti-PRRSV antibodies at 1 day of age even in the presence of maternally derived antibodies. The number of PRRSV-2 genomic copies in the sera of pigs from the PRRSV-2 MLV-vaccinated pigs was significantly (P<0.05) lower when compared to PRRSV-1 MLV-vaccinated pigs. The average daily gain in PRRSV-2 MLV-vaccinated pigs was significantly (P<0.05) higher when compared to both PRRSV-1 MLV-vaccinated and unvaccinated pigs. This study demonstrated that vaccination as early as 1 day of age was effective against PRRSV infection.

KEY WORDS: 1 day of age vaccination, modified live-virus vaccine, porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome (PRRS) (caused by the PRRS virus, or PRRSV), is recognized as the most economically devastating viral disease to the Korean swine industry. PRRSV belongs to the genus *Porartevirus* with two distinct species; PRRSV-1 and PRRSV-2, both of which are prevalent in Korean farms and can cause respiratory disease in growing pigs [10, 12, 14]. PRRSV modified-live virus (MLV) vaccines are the most common tool used to control PRRSV infection in Korean farms despite the limited protection offered against a heterologous challenge [8, 11, 12]. Currently, there are four commercially available PRRSV MLV vaccines in the Korean market, two based on PRRSV-1 and two based on PRRSV-2.

The timing of vaccination administration also plays an important role in the efficacy of a vaccine in order to induce the maximum protective immune response before the pig has a chance to become naturally infected. Recent data from Korean farms seem to suggest that the age of PRRSV infection in young piglets keeps increasing toward a younger age. In particular, the number of infected piglets between the ages of 4 and 6 weeks has increased significantly. Typically, PRRSV MLV vaccines are administered between the ages of 3 and 4 weeks, therefore it is unclear how well they would protect against PRRSV infection that occurs between 4–6 weeks of age. A commercially available PRRSV-2 MLV vaccine (Fostera^{TM}PRRS, Zoetis, Parsippany,

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NJ, USA) was recently licensed in Korea in 2017 for vaccination of 1-day-old piglets [6]. Since both PRRSV-1 and PRRSV-2 are prevalent in Korea, the objective of this study was to compare the efficacy of a PRRSV-1 and PRRSV-2 MLV vaccine when administered at 1 day of age under field conditions.

The clinical field trial was conducted on a two-site farm with 500-sows. In January 2017, five, 6-week-old pigs were submitted into the Department of Veterinary Pathology in Seoul National University to identify the cause of observed growth retardation. All five pigs were diagnosed with Glasser's disease as *Haemophilus parasuis* was isolated in fibrinous exudate in pericarditis. PRRSV-1 and PRRSV-2 was also isolated from both the tonsils and lungs. After consultation with the farm owner, it was decided to vaccinate future litters with a PRRSV MLV vaccine at 1 day of age.

The isolated PRRSV-1 field virus (SNUVR150266, GenBank MG271757) shared a 88.9% and 60.5% identity, when comparing the nucleotides of open reading frame 5 (ORF5), with the vaccine virus of UNISTRAIN PRRS (GenBank GU067771) and Fostera PRRS (GenBank AF 494042), respectively. The isolated PRRSV-2 field virus (SNUVR150267, GenBank MG385131) shared a 61.1% and 91.5% identity with the vaccine virus of UNISTRAIN PRRS (GenBank GU067771) and Fostera PRRS (GenBank AF 494042), respectively, based on the comparison of the nucleotides of ORF5. Despite the fact that ORF5 only covers 4% of the entire genome, it has been widely used for phylogenetic analysis because of its high genetic diversity [1].

A total of 120 colostrum-fed, cross-bred, conventional 1-day-old piglets were selected from fifteen healthy sows and divided into 3 groups (40 pigs per groups, 20 male and 20 female). Fifteen healthy pregnant sows (parity=1 or 2) at 7 days antepartum were randomly selected and allocated to groups for treatment and pen using the random number generator function (Excel, Microsoft Corp., Redmond, WA, USA). Sows were housed in individual crates with an empty crate between each sow to minimize the shedding of vaccine virus to controls from nose-to-nose contact. After farrowing, eight healthy newborn piglets (four male and four female) from each one of the 15 sows were selected and assigned into 3 groups using the random number generator function (Excel, Microsoft Corp.).

Pigs in the Vac1 group were intramuscularly injected with a 2.0 m*l* dose of a PRRSV-1 MLV vaccine (UNISTRAIN PRRS, Hipra, Lot No. 0L50) at 1 day of age. Pigs in the Vac2 group were intramuscularly injected with a 2.0 m*l* dose of a PRRSV-2 MLV vaccine (Fostera^{TM} PRRS, Zoetis, Lot No. 169588, Serial No. 163540/159469) at 1 day of age. Pigs in the UnVac group were intramuscularly injected with 2.0 m*l* of phosphate buffered saline (PBS, 0.01M, pH 7.4) at the same age.

All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee. Sample collection was carried out according to the animal welfare code of Korea.

At weaning (approximately 21 days of age), both vaccinated and unvaccinated pigs stayed on-site in their respective farm in accordance with the Korean field study protocol. They were housed by treatment (six pens per treatment and 4 pigs per pen within a barn) using the random number generator function (Excel, Microsoft Corp.). Pens were randomly assigned to litters and treatments with an empty pen between each occupied pen to minimize the shedding of the vaccine virus to controls through nose-to-nose contact. Blood samples were collected at 1, 7, 21, 35, 70, 91, and 112 days of age. The mortality rate was calculated as the number of pigs that died divided by the number of pigs initially assigned to a particular group.

The live weight of each pig from the Vac1, Vac2, and UnVac groups was measured at 1, 21, 70, 112, and 168 days of age. The average daily gain (ADG: defined as grams per pig per day) was analyzed over four time periods: between 1–21, 21–70, 70–112, and 112–168 days of age, respectively. The ADG during these various production stages was calculated as the difference between the starting and final weights divided by the duration of the stage. Data from dead pigs were excluded in the calculation.

RNA was extracted from serum samples to quantify the number of PRRSV genomic cDNA copies, as previously described [15]. Real-time PCR was performed to quantify PRRSV genomic cDNA copy for both PRRSV-1 and PRRSV-2 [15]. Real-time PCR was also used to quantify a PRRSV genomic cDNA copy for the vaccine viruses [5, 7, 13].

The serum samples were tested using a commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc., Westbrook, ME, USA). Serum samples were considered positive for PRRSV antibody if the S/P ratio was ≥0.4, according to the manufacturer's instructions. Serum virus neutralization tests were performed with the field virus as previously described [16, 17]. The presence of virus-specific cytopathic effect (CPE) in each well was recorded after 5 days of incubation. The presence of virus in wells without CPE was further determined by immunofluorescence microscopy using a SDOW17-FITC conjugate. Serum samples were considered to be positive for neutralizing antibody if the titer was greater than 2.0 ($\log_2$) [17].

The lungs were examined individually at slaughter. 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 [3]. The total amount of microscopic lung lesions was scored blindly by two pathologists with the lung sections ranging from 0 (normal) to 4 (severe). Lungs were also analyzed morphometrically using the NIH Image J 1.51r Program (http://imagej.nih.gov/ij/download.html) [3].

Statistical analysis was performed using SPSS software (version 21; IBM, Armonk, NY, USA). Continuous data included the following: ADG which was determined by the difference between the starting and final weights divided by the duration of the stage; PRRSV RNA ($\log_{10}$ of the number of PRRSV genomic copies per m*l*) was quantified by real-time PCR, and PRRSV antibody titer was measured by ELISA. Continuous data were analyzed using Tukey's multiple comparisons test for the comparison between groups in order to estimate the difference at each time point. Discrete data (clinical signs and lung lesion scores) were analyzed with the Kruskal-Wallis test. When the Kruskal-Wallis test was significant, the Mann-Whitney test was performed to determine the significant differences between the groups. A value of $P<0.05$ was considered significant. A linear regression was analyzed to determine the correlation between anti-PRRSV antibody titers at the day of vaccination (1 day of age) and the increase

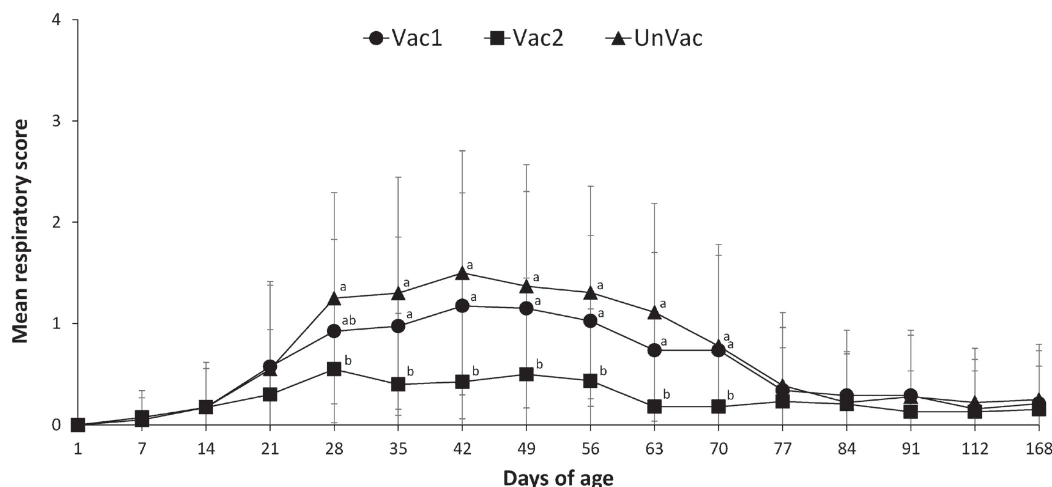

Fig. 1. Mean respiratory score in pigs from the Vac1 (●), Vac2 (■), and UnVac (▲) groups. Variation is expressed as the standard deviation. Different letters (a and b) indicate significant difference (*P*<0.05) among groups.

Table 1. Means (with standard deviation) of average daily gain (ADG) and lung lesion scores in pigs vaccinated for UNISTRAIN PRRS (Vac1 group), vaccinated for Fostera PRRS (Vac2 group), or injected with phosphate buffered saline (UnVac group) at 1 day of age

	Age (day)	Vac1	Vac2	UnVac
Vaccination	1	UNISTRAIN PRRS	Fostera PRRS	None
ADG (g/pig/day)	1–21	174.61 ± 34.38	180.51 ± 29.50	170.83 ± 35.76
	21–70	398.66 ± 71.41[b)]	447.67 ± 65.59[a)]	371.66 ± 80.13[b)]
	70–112	714.29 ± 111.54[a,b)]	772.89 ± 106.73[a)]	705.03 ± 137.04[b)]
	112–168	712.88 ± 98.69	686.36 ± 74.32	719.25 ± 87.64
	1–168	556.57 ± 25.77[b)]	577.51 ± 26.19[a)]	548.00 ± 29.13[b)]
Lung lesion score				
Macroscopic	168	16.32 ± 13.99	13.21 ± 11.63	19.17 ± 14.26
Microscopic	168	1.03 ± 0.74	0.92 ± 0.76	1.28 ± 0.87

Different letters (a and b) indicate significant difference (*P*<0.05) among groups.

of anti-PRRSV antibody titers at 34 days post vaccination (35 days of age: delta value, defined as anti-PRRSV antibody titers at 34 days post vaccination minus PRRSV antibody titers at the day of vaccination).

The mean respiratory scores were significantly (*P*<0.05) lower in pigs from the Vac2 group compared to pigs from the UnVac group at 28 days of age. The mean respiratory scores were significantly (*P*<0.05) lower in pigs from the Vac2 group when compared to pigs from the Vac1 and UnVac groups at 35, 42, 49, 56, 63, and 70 days of age (Fig. 1).

Two pigs from the Vac1 group died of Glasser's disease caused by *H. parasuis* at 52 days of age. One pig from the Vac2 group also died of Glasser's disease caused by *H. parasuis* at 55 days of age. Two pigs died from the UnVac group of pleuropneumonia caused by *Actinobacillus pleuropneumoniae* at 55 days of age while two pigs from the UnVac group died of Glasser's disease caused by *H. parasuis* at 49 days of age.

Seven lung samples were collected from 7 pigs within 3 groups that died during the field study and screened by PCR. Five of the samples were positive for PRRSV-1 and six were positive for PRRSV-2. A comparison of the ORF5 sequences of the PRRSV-1 and PRRSV-2 positive samples with PRRSV strains isolated prior to vaccination revealed only a 98.3–100% and 98.5–100% identity match with field PRRSV-1 (SNUVR150266, GenBank MG271757) and PRRSV-2 (SNUVR150267, GenBank MG385131) viruses, respectively.

The ADG was significantly (*P*<0.05) higher in pigs from the Vac2 group compared to pigs from the Vac1 and UnVac groups between 21 and 70 days of age. The ADGs were significantly (*P*<0.05) higher in pigs from the Vac2 group when compared with pigs from the UnVac group between 70 and 112 days of age. The overall ADG (from 1 to 168 days of age) in pigs from the Vac2 group was significantly (*P*<0.05) higher compared to pigs from the Vac1 and UnVac groups (Table 1).

The number of PRRSV-1 genomic copies from the PRRSV-1 MLV vaccine virus of the Vac1 pigs was detected in the serum of 40 pigs at 7 days of age and in 12 pigs at 21 days of age. The number of PRRSV-2 genomic copies from the PRRSV-2 MLV vaccine virus of the Vac2 pigs was detected in the serum of 40 pigs at 7 days of age and in 11 pigs at 21 days of age. Vaccine virus was not detected in the serum of pigs from the Vac1 and Vac2 groups at 35 days of age. Cross-contamination of the vaccine virus was not observed between the two vaccinated groups (Vac1 and Vac2). Vaccine virus was not detected in the serum of pigs from

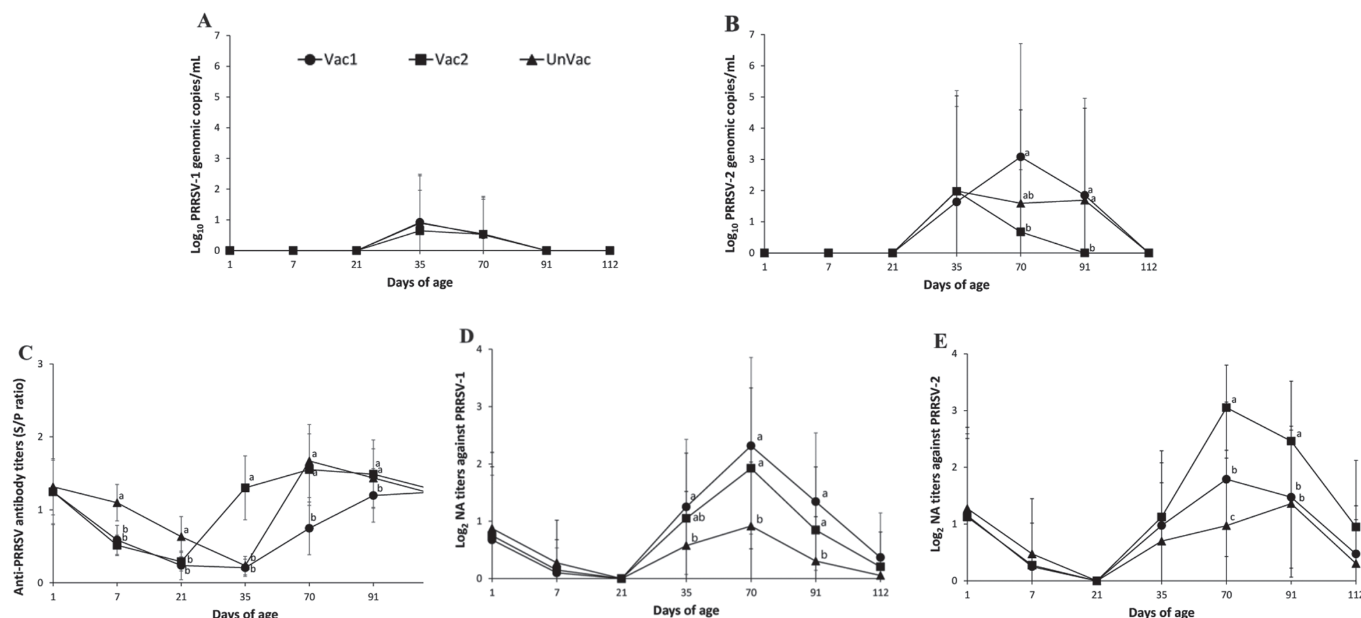

Fig. 2. Mean values of the genomic copy number of porcine reproductive and respiratory syndrome virus (PRRSV)-1 RNA in serum (A), the genomic copy number of PRRSV-2 RNA in serum (B), the anti-PRRSV antibody titers (C), neutralizing antibody titers against PRRSV-1 (D), and neutralizing antibody titers against PRRSV-2 (E) in serum of pigs from the Vac1 (●), Vac2 (■), and UnVac (▲) groups. Variation is expressed as the standard deviation. Different letters (a, b and c) indicate significant difference (*P*<0.05) among groups.

the UnVac group throughout the study.

A difference between pigs from the Vac1, Vac2, and UnVac groups was not found in the number of PRRSV-1 genomic copies in the sera throughout the experiment (Fig. 2A). The number of PRRSV-2 genomic copies in the sera of pigs from the Vac2 group was significantly (*P*<0.05) lower compared to pigs from the Vac1 group at 70 days of age. The number of PRRSV-2 genomic copies in the sera of pigs from the Vac2 group was also significantly (*P*<0.05) lower compared to pigs from the Vac1 and UnVac groups at 91 days of age (Fig. 2B).

Anti-PRRSV antibody titers were significantly (*P*<0.05) higher in pigs from the UnVac group when compared with pigs from the Vac1 and Vac2 groups at 7 and 21 days of age. Anti-PRRSV antibody titers were significantly (*P*<0.05) higher in pigs from the Vac2 group when compared with pigs from the Vac1 and UnVac groups at 35 days of age. Anti-PRRSV antibody titers were significantly (*P*<0.05) higher in pigs from the Vac2 and UnVac groups when compared with pigs from the Vac1 group at 70 and 91 days of age (Fig. 2C). The results from this study suggested a negative correlation between anti-PRRSV antibody titers and vaccination of pigs with the PRRSV-1 (r= −0.968, *P*<0.05) and PRRSV-2 MLV vaccines (r= −0.829, *P*<0.05).

Neutralizing antibody titers against PRRSV-1 were significantly (*P*<0.05) higher in pigs from the Vac1 group when compared with pigs from the UnVac group at 35 days of age. Neutralizing antibody titers against PRRSV-1 were significantly (*P*<0.05) higher in pigs from the Vac1 and Vac2 groups when compared with pigs from the UnVac group at 70 and 91 days of age (Fig. 2D). Neutralizing antibody titers against PRRSV-2 were significantly (*P*<0.05) higher in pigs from the Vac2 group when compared with pigs from the Vac1 and UnVac groups at 70 and 91 days of age. Neutralizing antibody titers against PRRSV-2 were significantly (*P*<0.05) higher in pigs from the Vac1 group when compared with pigs from the UnVac group at 70 days of age (Fig. 2E).

The results presented here demonstrate that the PRRSV-2 MLV vaccine is more efficacious than the PRRSV-1 MLV vaccine in controlling a co-infection of PRRSV-1 and PRRSV-2 while administrating vaccine to 1-day-old piglets under field conditions. One of the most important parameters in assessing the efficacy of a PRRSV MLV vaccine under field conditions is growth performance. In this field trial, pigs that were vaccinated with a PRRSV-2 MLV vaccine exhibited a better weight gain when compared to unvaccinated pigs or pigs that were vaccinated with a PRRSV-1 MLV vaccine. The difference in growth performance between the two PRRSV MLV vaccines appears to correlate with the difference in reduction of PRRSV-1 and PRRSV-2 viremia. The PRRSV-2 MLV vaccine was able to reduce PRRSV-2 but was unsuccessful in reducing PRRSV-1 viremia. However, the PRRSV-1 MLV vaccine failed to reduce both PRRSV-1 and PRRSV-2 viremia. In Korea, PRRSV-2 is more virulent compared to PRRSV-1 [4] and the control of PRRSV-2 infection has a bigger impact on growth performance in farms which are co-infected with PRRSV-1 and PRRSV-2. The lack of a significant difference in growth performance between PRRSV-1 MLV vaccinated pigs and unvaccinated pigs suggest that the PRRSV-1 MLV vaccine is not effective enough against co-infection with PRRSV-1 and PRRSV-2. However, these data should be interpreted with caution because the PRRSV-1 MLV vaccine used in this study was administered off label to piglets at 1 day of age. Therefore, the lack of growth performance improvement could be due to the fact that the vaccine was administered too early. Although two PRRSV-1 MLV vaccines exist in Korea, the one selected for this study was done so as it claims protection against both genotypes (www.hipra.com). Neither vaccine, however, recommends vaccination at 1 day of age.

The biggest concern with PRRSV vaccination at 1 day of age is the interference that may be caused by maternally derived antibodies. In general, pigs with high maternally derived NA (~7.0 log_2) interfere with the development of the humoral immune response after PRRSV vaccination [2]. Within this field study the effect of maternally derived NA against PRRSV vaccination at 1 day of age is unclear due to the low levels of maternally derived NA (2 to 3 log_2) at the time of vaccination. In addition, pigs aged between 21 and 35 days of age had already become infected with field PRRSV in this farm. It should be noted that maternally derived ELISA antibodies were able to interfere with the active induction of anti-PRRSV ELISA antibodies by either of the MLV vaccines. Currently, evidence does not exist to support that PRRSV antibodies detected by ELISA play a role in protection against infection with PRRSV [9]. Despite the interference with maternally derived antibodies, PRRSV-2 MLV-vaccinated pigs did exhibit better growth performance when compared with unvaccinated pigs. Therefore, PRRSV-2 MLV-vaccination at 1 day of age seems to improve growth performance even in the presence of the maternally derived antibodies.

It is recommended that a PRRSV vaccine should be administered prior to animals coming into contact with PRRSV. The farm used in this field trial was a typical farm where vaccination is usually performed at 3 or 4 weeks of age according to the manufacturer's recommendation. However, based on recent data this may be too late since piglets are becoming infected with PRRSV-1 and PRRSV-2 as early as 5 weeks of age. The results presented in this field study suggest that it is possible for swine producers to vaccinate as early as 1 day old in order to prevent PRRSV infection around 5 weeks of age.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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