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Efficacy of concurrent vaccination with modified-live PRRSV-1 and PRRSV-2 vaccines against heterologous dual PRRSV-1 and PRRSV-2 challenge in late term pregnancy gilts

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ABSTRACT

The objective of this study was to evaluate the effect of concurrent vaccination with a porcine reproductive and respiratory syndrome virus (PRRSV)-1 modified-live virus (MLV) vaccine and a PRRSV-2 MLV vaccine against a dual heterologous PRRSV-1 and PRRSV-2 challenge in late term pregnancy gilts. Gilts were concurrently administered PRRSV-1 and PRRSV-2 MLV vaccines at 21 days prior to breeding at separate anatomical sites and were inoculated intranasally with both PRRSV types at 93 days of gestation. Vaccinated gilts had a higher number of live-born and weaned pigs, and a decrease in stillbirths compared to the unvaccinated control group following a dual challenge. Concurrent vaccination resulted also in the reduction of both PRRSV-1 and PRRSV-2 viremia which correlated with an increase in the number of PRRSV-1 and PRRSV-2 specific interferon-γ secreting cells (IFN-γ-SC). We believe the T cell responses contributed to the reduction of both PRRSV-1 and PRRSV-2 viremia. The results presented here demonstrate that concurrent vaccination with PRRSV-1 and PRRSV-2 MLV vaccines improves reproductive performance, reduces viremia of PRRSV-1 and PRRSV-2, and induces protective T cell reactions against dual PRRSV-1 and PRRSV-2 challenge in late term pregnancy gilts without local and systemic adverse reactions related to concurrent vaccination.

1. Introduction

The porcine reproductive and respiratory syndrome (PRRS) is one the most economically important viral diseases that has been impacting the global swine industry for almost 30 years since it was first described in the late 1980s in North America. The etiological agent, PRRS virus (PRRSV), is an RNA virus that belongs to the *Porarterivirus* genus, *Arteriviridae* family, and order *Nidovirales* (Adams et al., 2016). PRRSV is classified into two major species referred to as PRRSV-1 (European origin) and PRRSV-2 (North American origin) (Adams et al., 2016; Allende et al., 1999; Forsberg et al., 2002; Murtaugh et al., 2010). PRRSV-1 and PRRSV-2 are the predominant species in European and North American continents, respectively, however for most Asian countries including Korea the situation is more complicated because both PRRSV-1 and PRRSV-2 are prevalent and have been shown to cause disease (Choi et al., 2015). PRRS is characterized by two distinct clinical presentations, reproductive failure in gilts and sows, and respiratory disorders predisposing growing pigs to secondary infections associated with the porcine respiratory disease complex. The clinical signs of reproductive failure in breeding animals are characterized by third-trimester abortions and premature farrowing along with increased incidence of fetal mummies, stillborn, or weak-born piglets, and neonatal death (Christianson et al., 1993; Zimmerman et al., 2012).

In Korea, PRRSV modified-live virus (MLV) vaccines are the main defense tool used to control PRRSV spread throughout the sow population within a herd. Currently, there are two types of commercially available PRRSV MLV vaccines in Korea; one is an MLV vaccine based on PRRSV-1 and the second is an MLV vaccine based on PRRSV-2. In recent years, diagnostic cases of aborted fetuses have revealed an increase in coinfection with PRRSV-1 and PRRSV-2 (Jeong et al., 2018b). For example, in 2018, both species were detected in 48% of aborted fetuses (13 out of 27 PRRSV-positive aborted cases), an increase of more than 50% compared to the previous two years (24%, 12 out of 54 PRRSV-positive aborted cases). These data are increasingly underscoring the need for tools to control both PRRSV-1 and PRRSV-2 at the same times. Although we have previously shown that a PRRSV-2 MLV vaccine can cross-protect against PRRSV-1 in terms of reproductive failure (Jeong et al., 2018c), a PRRSV-1 MLV vaccine always provides

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better protection (Jeong et al., 2018a, 2018b). On the other hands, the PRRSV-1 MLV vaccine provides no protection against reproductive failure caused by PRRSV-2 in late-term pregnancy gilts while the PRRSV-2 MLV vaccine is very effective (Jeong et al., 2018a, 2018b). Taken together, these results suggest that PRRSV MLV vaccines protect better against a challenge with the same species as the vaccine strain.

Based on our previous publications (Jeong et al., 2018a, 2018b), theoretically, concurrent vaccination with PRRSV-1 and PRRSV-2 MLV vaccines may provide complete protection against reproductive failure from PRRSV-1 and PRRSV-2 in sows and gilts. However, no studies have been done yet to test the efficacy of concurrent vaccination to protect sows from reproductive failure caused by a coinfection with PRRSV-1 and PRRSV-2. The objective of this study was to determine how efficacious concurrent vaccination with PRRSV-1 and PRRSV-2 MLV vaccines would be against dual heterologous PRRSV-1 and PRRSV-2 challenge in late term pregnancy gilts.

2. Materials and methods

2.1. PRRSV isolates

PRRSV-1 (SNUVR090485, pan-European subtype 1) and PRRSV-2 (SNUVR090851, lineage 1) were used as inocula (Han et al., 2011, 2014). The vaccine virus shares 88.1% and 59.3% nucleotide sequence identity for open reading frame 5 (ORF5) with PRRSV-1 (GenBank no. JN315686) and PRRSV-2 (GenBank no. JN315685) challenge strains, respectively.

2.2. Experimental design

Eighteen clinically healthy, cross-bred, gilts were purchased from a commercial PRRSV free farm that had not been vaccinated against PRRSV. Gilts were negative for PRRSV and porcine circovirus type 2 (PCV2) as tested by antibody ELISA and real-time polymerase chain reaction (RT-PCR) (Gagnon et al., 2008; Wasilk et al., 2004). Eighteen gilts were randomly assigned into 3 groups (6 gilts per group) using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA) which were housed in separate rooms (Table 1). Each room contained 6 pens and each individual gilt was randomly assigned to an individual pen.

At —114 days post challenge (dpc, 3 weeks prior to breeding), gilts in Vac1-2/Ch1-2 group were intramuscularly injected with a 2.0 mL dose of PRRSV-1 MLV vaccine (UNISTRAIN PRRS, Lot No. 0L50) at left side of the neck and a 2.0 mL dose of PRRSV-2 MLV vaccine (Fostera™PRRS, Zoetis, Lot No. 169588, Serial No. 163540/159469, Zoetis) at right side of the neck. Gilts in UnVac/Ch1-2 and UnVac/UnCh groups were intramuscularly injected with 2.0 mL of phosphate buffered saline (PBS, 0.01 M, pH 7.4).

At 0 dpc (93 days of gestation), pregnant gilts in Vac1-2/Ch1-2 and UnVac/Ch1-2 groups were inoculated intranasally with 6 mL of each PRRSV-1 and PRRSV-2 inoculums (10^4 $TCID_{50}$/mL of SNUVR090485, second passage in alveolar macrophages and 10^4 $TCID_{50}$/mL of SNUVR090851, second passage in alveolar macrophages, respectively). Pregnant gilts in UnVac/UnCh group were inoculated similarly with PBS.

Upon challenge, pregnant gilts in the Vac1-2/Ch1-2 and UnVac/Ch1-2 groups were randomly assigned into 2 out of 3 rooms. Each room contained 6 pens and each pregnant gilt was housed individually in a pen. In each of the 2 rooms, allocation of pens to treatment was randomly assigned. Pregnant gilts in the UnVac/UnCh group were randomly placed into 6 pens in the one remaining room.

2.3. Reproductive performance

The pregnant gilts were monitored daily for rectal temperature after challenge by the same personnel. Farrowing data, including litter size [piglet total number, live birth, stillborn, mummified, and light (< 1 Kg body weight) per litter] at birth and piglet weaned at 21 days of age, were also recorded.

2.4. Quantification of PRRSV RNA in blood

Serum samples from pregnant gilts were collected and RNA was extracted as previously described (Han et al., 2011; Wasilk et al., 2004). The primers for both challenge virus were designed based on the highly conserved ORF7 region. For the PRRSV-1, the forward and reverse

Table 1
Reproductive parameters (mean ± standard deviation) of gilts among 3 groups.

		Vac1-2/Ch1-2			UnVac/Ch1-2			UnVac/UnCh		
Vaccination		PRRSV-1 & -2			None			None		
Challenge		PRRSV-1 & -2			PRRSV-1 & -2			None		
Gilts										
	Gestation length	113.83	±	1.07 [a]	104.67	±	1.97 [b]	114.50	±	0.50 [a]
	Premature farrowing	0/6			6/6			0/6		
Piglets/litter										
	Total born	12.50	±	1.12	12.67	±	1.89	12.83	±	1.34
	Live-born	11.67	±	1.11 [a]	2.67	±	0.75 [b]	12.50	±	1.38 [a]
	Stillborn	0.83	±	0.69 [b]	9.33	±	1.11 [a]	0.33	±	0.47 [b]
	Mummified	0	±	0	0.67	±	0.75	0	±	0
	Light (< 1 Kg)	0.33	±	0.47	0.50	±	0.50	0.33	±	0.47
	Splay-legs	0.17	±	0.37	0.33	±	0.47	0.17	±	0.37
	Weaned	11.00	±	1.15 [a]	2.50	±	0.76 [b]	11.67	±	1.11 [a]
Fetal PRRSV-1 in situ hybridization score										
	Lung	0.20	±	0.40	0.39	±	0.65	0	±	0
	Lymph node	0.40	±	0.49	1.59	±	1.46	0	±	0
	Thymus	1.20	±	1.17 [b]	9.82	±	2.87 [a]	0	±	0 [b]
	Tonsil	0.40	±	0.49	1.79	±	1.32	0	±	0
	Heart	0.20	±	0.40	0.34	±	0.61	0	±	0
Fetal PRRSV-2 in situ hybridization score										
	Lung	8.00	±	3.03 [a]	8.14	±	3.57 [a]	0	±	0 [b]
	Lymph node	16.00	±	3.16 [a]	19.30	±	3.22 [a]	0	±	0 [b]
	Thymus	34.60	±	6.09 [b]	43.91	±	7.61 [a]	0	±	0 [c]
	Tonsil	16.80	±	2.40 [b]	21.16	±	3.35 [a]	0	±	0 [c]
	Heart	4.40	±	2.06 [a]	4.98	±	1.99 [a]	0	±	0 [b]

Different superscripts (a, b, and c) indicate significant ($P < 0.05$) difference among 3 groups.

primers were 5′- TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTG CAAGCAGAGGGAA-3′, respectively. For PRRSV-2, the forward and reverse primers were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATC GATTGCAAGCAGAGGGAA-3′, respectively (Wasilk et al., 2004). For UNISTRAIN vaccine virus, the forward and reverse primers were 5′-GTTGCCCAGCCATTTTGAC-3′ and 5′-CACGCTGCTGAGTACATACC-3′, respectively (Kim et al., 2015). For Fostera PRRS vaccine virus, the forward and reverse primers were 5′-CTTGACACAGTTGGTCTGGTT ACT-3′ and 5′-GTTCTTCGCAAGCCTAATAACG-3′, respectively (Park et al., 2014). Real-time PCR for PRRSV-1 and PRRSV-2 was used to quantify PRRSV genomic cDNA copy numbers using RNA extracted from serum samples as previously described (Park et al., 2014; Wasilk et al., 2004).

2.5. Serology

The serum samples were tested for total PRRSV-specific antibodies using a commercially available PRRSV 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 also performed with challenge PRRSV-1 (SNUVR090485 strain) and PRRSV-2 (SNUVR090851 strain), as previously described (Wu et al., 2001; Zuckermann et al., 2007). Serum samples were considered to be positive for neutralizing antibody if the titer was greater than 2.0 ($\log_2$) (Zuckermann et al., 2007).

2.6. Enzyme-linked immunospot (ELISPOT) assay

The numbers of PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) were measured in peripheral blood mononuclear cells (PBMC) as previously described (Diaz et al., 2005; Meier et al., 2003) with some modifications. Briefly, 100 µL of RPMI 1640 medium supplemented with 10% fetal bovine serum (HyClone Laboratories, Inc., SelectScience, Bath, UK), 1 mM non-essential amino acids (Invitrogen, Carlsbad, CA, USA), 1 mM sodium pyruvate, 5 mM 2-mercaptoethanol, 50,000 IU/L penicillin l, and 50 mg/L streptomycin, containing 5×10^5 PBMC was seeded into each well of plates that were precoated overnight at 4 °C with anti-porcine IFN-γ monoclonal antibody (10 µg/mL, MABTECH, Mariemont, OH, USA). The cells were stimulated with 100 µL of either challenge PRRSV-1 or PRRSV-2 strain diluted in RPMI 1640 medium (10^6 $TCID_{50}$/mL) for 20 h at 37 °C in a 5% humidified CO_2 atmosphere. The linear response was tested at a multiplicity of infection (MOI) between 0.01 and 0.1. Phytohemagglutinin (10 µg/mL, Roche Diagnostics GmbH, Mannheim, Germany) and culture medium were used as positive and negative control, respectively. Next, the wells were washed five times with PBS (200 µL per well). Thereafter, the procedure was conducted according to the manufacturer's instructions using the commercial ELISPOT Assay Kit (MABTECH). In a typical PRRSV-specific reaction, the background caused by the non-PRRSV stimulated wells (negative control) did not exceed five spots per cell and the obtained values were subtracted from the respective counts obtained from the stimulated wells. The IFN-γ positive spots on the membranes were imaged, analyzed and counted using an automated ELISPOT Reader (AID ELISPOT Reader, AID GmbH, Strassberg, Germany). ELISPOT assay was repeated twice.

2.7. In situ hybridization

In situ hybridization (ISH) for the detection of PRRSV-1 and PRRSV-2 nucleic acid in fetal tissues from stillborn piglets was performed and analyzed morphometrically as previously described (Halbur et al., 1996; Han et al., 2013).

2.8. Statistical analysis

Prior to statistical analysis, RT-PCR and neutralizing antibody data were transformed to $\log_{10}$ and $\log_2$ values, respectively. Data was tested for normal distribution using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used to examine whether there were statistically significant differences among the four groups, for each time point. When a test result from one-way ANOVA showed a statistical significance, a post-hoc test was conducted for a pairwise comparison with Tukey's adjustment. If the normality assumption was not met, the Kruskal-Wallis test was performed. When the result from Kruskal Wallis test showed statistical significance, Mann-Whitney test with Tukey's adjustment was performed to compare the differences among the groups. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Concurrent vaccinated challenged gilts improved reproductive performance

To evaluate the reproductive performance we compared gestation periods, and the number of live-born, weaned and stillborn piglets between the vaccinated and control groups. Pregnant gilts from the UnVac/Ch1-2 group had premature farrowing with significantly shorter ($P < 0.05$) gestation periods compared to the Vac1-2/Ch1-2 and UnVac/UnCh groups which had normal farrowing. In addition, pregnant gilts from the Vac1-2/Ch1-2 and UnVac/UnCh groups had a significantly higher ($P < 0.05$) number of live-born and weaned piglets and had a significantly lower ($P < 0.05$) number of stillborn piglets compared to the UnVac/Ch1-2 group (Table 1). No adverse systemic or local reactions related to vaccination were observed throughout the entire gestation period.

3.2. Concurrent vaccinated challenged gilts reduced PRRSV viremia in sera

The presence of PRRSV RNA in the blood was quantified using real-time quantitative PCR. At the time of vaccination (–114 dpc, 3 weeks prior to breeding) and challenge (0 dpc, 93 days of gestation) no genomic copies of either PRRSV-1 or PRRSV-2 could be detected in the serum of all pregnant gilts from all three groups. At 7 dpc, gilts from the Vac1-2/Ch1-2 group had a significantly ($P < 0.05$) lower number of genomic copies of PRRSV-1 RNA in their sera compared to the UnVac/Ch1-2 group (Fig. 1A). In addition, at 7and 21 dpc, gilts from the Vac1-2/Ch1-2 group had a significantly lower ($P < 0.05$) number of genomic copies of PRRSV-2 RNA in their sera compared to the UnVac/Ch1-2 group (Fig. 1B). Neither PRRSV-1 nor PRRSV-2 RNA was detected in the sera of gilts from the UnVac/UnCh group throughout the course of this study.

3.3. Concurrent challenged gilts elicited ELISA and serum neutralization antibodies

At the time of PRRSV vaccination (–114 dpc, 21 days prior to breeding), pregnant gilts in all 3 groups were seronegative. At –93, –65, 0, 7, and 21 dpc, gilts from the Vac1-2/Ch1-2 group had a significantly higher ($P < 0.05$) ELISA S/P ratio compared to the UnVac/Ch1-2 group (Fig. 2). No anti-PRRSV antibodies were detected by ELSIA in gilts from the UnVac/UnCh group throughout the experiment.

At –93, –65, 0, 7, and 21 dpc, gilts from the Vac1-2/Ch1-2 group had significantly higher ($P < 0.05$) neutralizing antibody titers against PRRSV-1 (Fig. 3A) and PRRSV-2 (Fig. 3B) compared to the UnVac/Ch1-2 group. No neutralizing antibodies against either PRRSV-1 or PRRSV-2 were detected in pregnant gilts from the UnVac/UnCh group throughout the experiment.

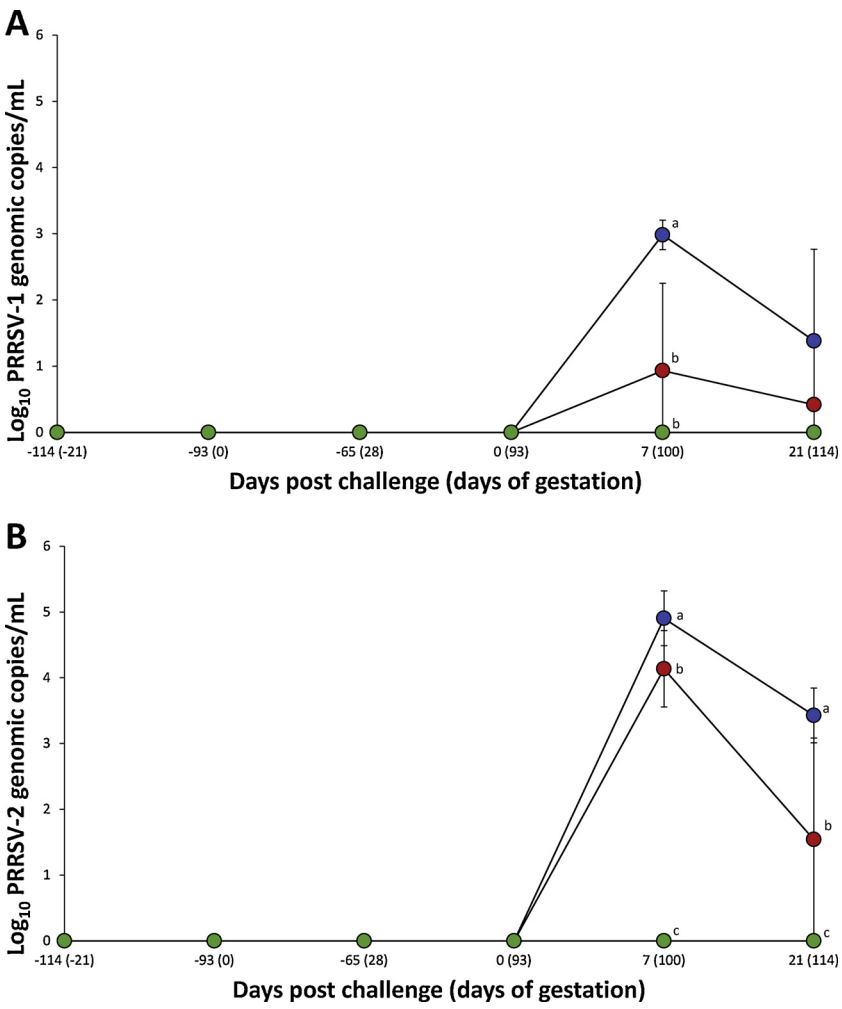

Fig. 1. Mean values of the genomic copy number of PRRSV − 1 (A) and PRRSV − 2 (B) RNA in serum from Vac1 − 2/Ch1 − 2 (●), UnVac/Ch1 − 2 (●), UnVac/UnCh (●) groups. Variation is expressed as the standard deviation. Different superscripts (a, b, and c) indicate significant ($P < 0.05$) difference among 3 groups.

3.4. Concurrent vaccinated challenged gilts induced interferon-γ secreting cells

T cell response was evaluated by looking at the induction of PRRSV specific IFN-γ-SC. At –93, –65, 0, 7, and 21 dpc, pregnant gilts from the Vac1-2/Ch1-2 group had a significantly higher ($P < 0.05$) number of PRRSV-1 (Fig. 4A) and PRRSV-2 (Fig. 4B) specific IFN-γ-SC compared to the UnVac/Ch1-2 group. No PRRSV-1 or PRRSV-2 specific IFN-γ-SC was detected in gilts from the UnVac/UnCh group throughout the experiment.

3.5. Concurrent vaccinated challenged gilts reduced fetal PRRSV nucleic acids

Tissues from stillborn fetuses were stained to confirm the presence of PRRSV-1 and PRRSV-2. Stillborn fetuses from gilts in the Vac1-2/Ch1-2 group had a significantly lower ($P < 0.05$) number of PRRSV-1 positive cells in thymus compared to the UnVac/Ch1-2 group. Stillborn fetuses from pregnant gilts in the Vac1-2/Ch1-2 group also showed a significantly lower ($P < 0.05$) number of PRRSV-2 positive cells in the thymus and tonsils compared to the UnVac/Ch1-2 group (Fig. 5A and B). No PRRSV-1 or PRRSV-2 positive cells were detected in any of the

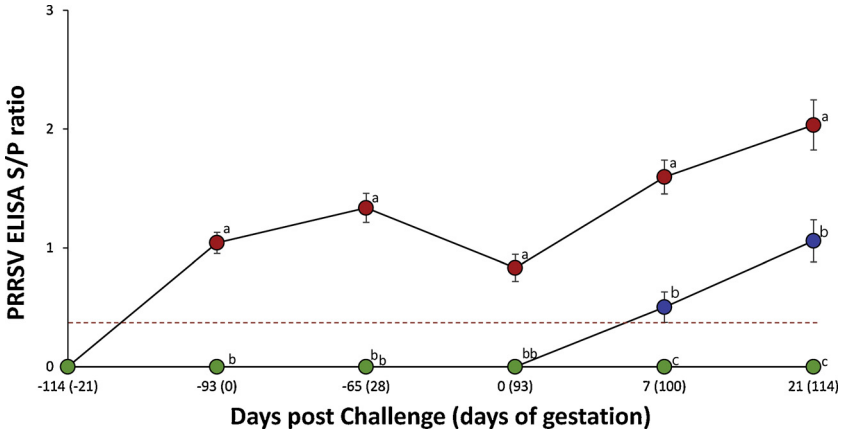

Fig. 2. Mean values of the PRRSV ELISA S/P ratio from Vac1-2/Ch1-2 (●), UnVac/Ch1-2 (●), UnVac/UnCh (●) groups. Variation is expressed as the standard deviation. Serum samples are considered to be positive for anti-PRRSV antibody if the ELISA S/P ratio is ≥ 0.4 (red dotted line). Different superscripts (a, b, and c) indicate significant ($P < 0.05$) difference among 3 groups (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

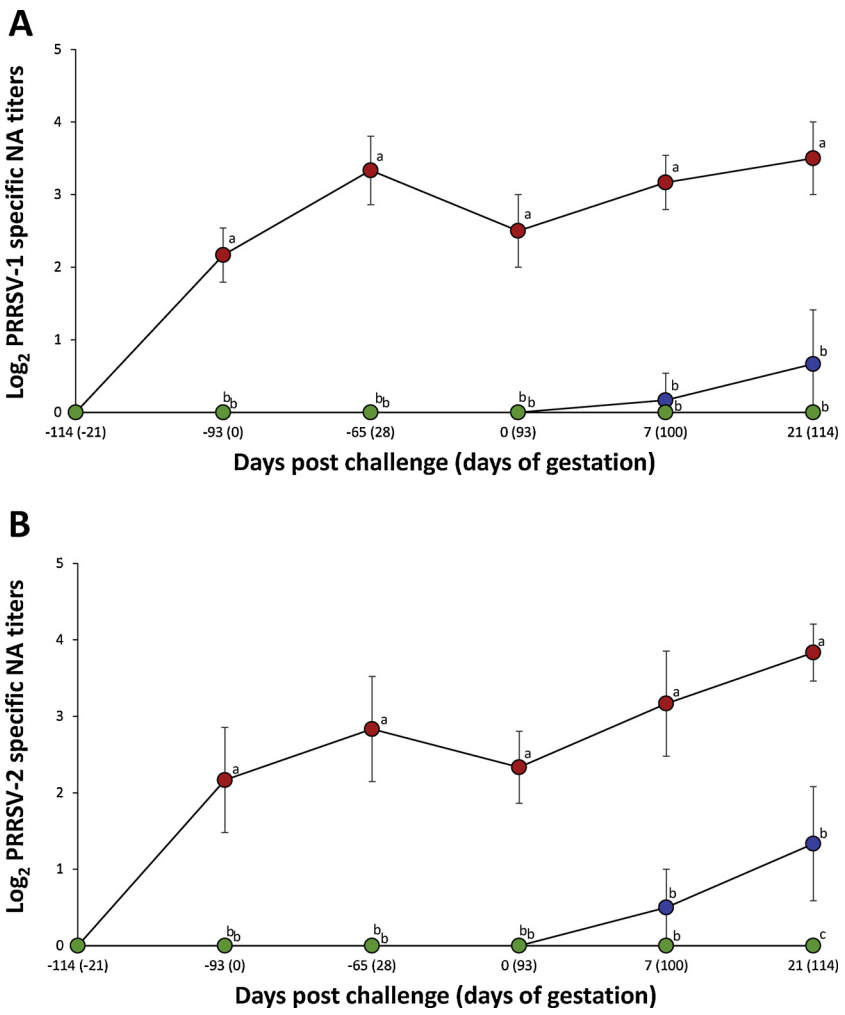

Fig. 3. Neutralizing antibody (NA) titers against PRRSV − 1 (A) and PRRSV − 2 (B) from Vac1 − 2/Ch1 − 2 (●), UnVac/Ch1 − 2 (●), UnVac/UnCh (●) groups. Variation is expressed as the standard deviation. Different superscripts (a, b, and c) indicate significant ($P < 0.05$) difference among 3 groups.

fetal tissues of pregnant gilts from the UnVac/UnCh group throughout the study.

4. Discussion

The results of this study that we have presented here demonstrate that concurrent vaccination with a PRRSV-1 and PRRSV-2 MLV vaccine provides good protection against reproductive failure caused by dual heterologous PRRSV-1 and PRRSV-2 challenge in late-term pregnancy gilts. To avoid the possibility of interference, the vaccines were administered at different anatomical sites. Loss of reproductive performance is one of the main reasons PRRSV infection has such a severe economic impact on swine farms. In our study, concurrent vaccination with PRRSV-1 and PRRSV-2 MLV vaccines resulted in an increase of live-born and weaned piglets and a decrease in stillbirths, significantly improving the reproductive performance. Protection with co-vaccination against dual heterologous challenge was similar to results from previous studies with single vaccinations with a PRRSV-1 or PRRSV-2 MLV vaccine against single challenge with PRRSV-1 or PRRSV-2 respectively.

Our results are also consistent with a previous study, where co-vaccinated boars resulted in reduction of seminal shedding and viremia of PRRSV-1 and PRRSV-2 after dual PRRSV-1 and PRRSV-2 challenge (Jeong et al., 2017). Protection against respiratory disease caused by dual challenge has been reported with mixed results. Co-vaccination of pigs at 4 weeks of age provided only partial protection against respiratory disease caused by dual challenge with PRRSV-1 and PRRSV-2 (Kristensen et al., 2018) whereas another study reported that concurrent vaccination could only provide protection against respiratory disease caused by PRRSV-1 in 4-week-old pigs with dual challenge (Park et al., 2015). There are two possibilities for the discrepant results. First, the two studies that performed concurrent vaccinations in 4-week-old pigs used different commercial PRRSV-2 MLV vaccines. The vaccine strains of the two PRRSV-2 MLV vaccines are genetically distant; one is lineage 5 and the other is lineage 9. These genetic differences could play a role on the efficacy of the vaccine. In addition, the two studies also used different challenge strains of PRRSV-1 and PRRSV-2. The efficacy of PRRSV MLV vaccine could be different depending on the challenge strain (Kim et al., 2015; Ko et al., 2016). Second, the different outcomes could be due to age-related immune responses. It appears that concurrent vaccination is more efficacious in adult pigs (i.e., gilts and boar) because their immune systems may be mature enough to have a simultaneous immune response to each vaccine. Weaning pigs at younger than 5 weeks old can cause physiological changes that can be detrimental to cellular immune responses (Blecha et al., 1983). Similarly, the age of the host can influence the dynamics of PRRSV infection (Klinge et al., 2009). Further studies are needed to elucidate these hypotheses.

Maternal viremia also plays an important role in reproductive failure. In the early stages of infection PRRSV is able to cross the placenta and initially infect only a few fetuses. This transplacental infection correlates with levels of maternal viremia (Ladinig et al., 2015a; Novakovic et al., 2017). Subsequently, infection then spreads from fetus to fetus independently of maternal viremia (Ladinig et al., 2015a, 2015b; Harding et al., 2017). Therefore, the ability of a vaccine to reduce maternal viremia especially early on during PRRSV infection is

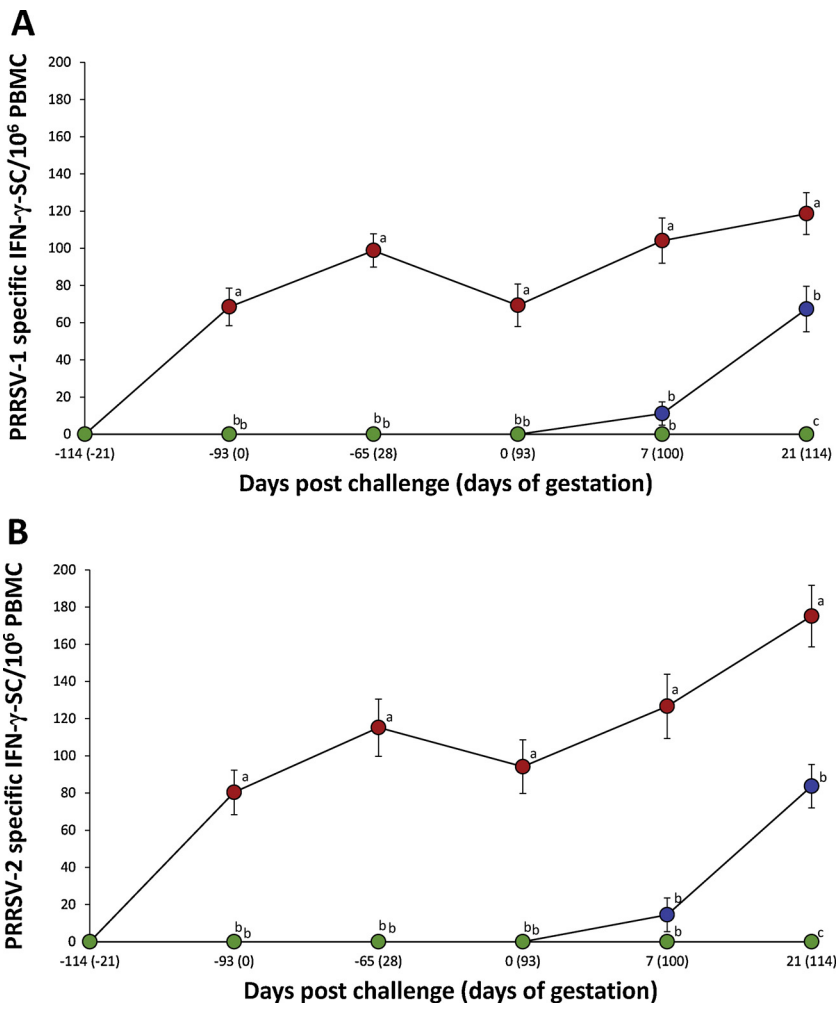

Fig. 4. Frequency of PRRSV-1 (A) and PRRSV-2 (B) specific IFN-γ-SC/10^6 PBMC from Vac1 − 2/Ch1 − 2 (●), UnVac/Ch1 − 2 (●), UnVac/UnCh (●) groups. Variation is expressed as the standard deviation. Different superscripts (a, b, and c) indicate significant ($P < 0.05$) difference among 3 groups.

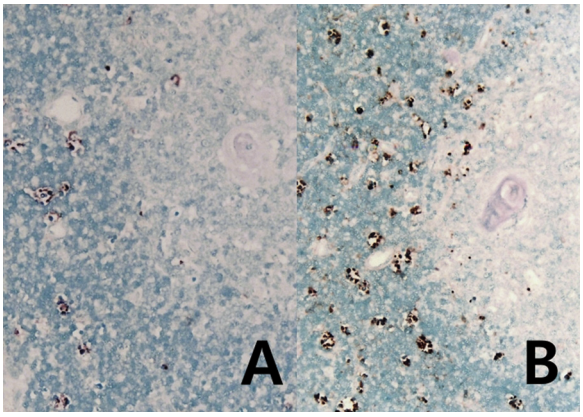

Fig. 5. In situ hybridization. PRRSV-2 nucleic acids were detected in the fetal thymus from the Vac1-2/Ch1-2 (A) and UnVac/Ch1-2 (B) groups by in situ hybridization.

critical in controlling reproductive failure. In our study, pregnant gilts that were co-vaccinated experienced a significant reduction in the amount of PRRSV-1 or PRRSV-2 viral load in their blood as early as 7 dpc. The early reduction of viremia could explain why co-vaccination provides good protection against the dual challenge. Once PRRSV crosses the placenta and infects the fetus, the amount of viral load in feta thymus of the fetus is a determining factor in fetal death. The higher the viral load in the fetal thymus, the higher the likelihood of fetal death (Ladinig et al., 2015a; Novakovic et al., 2017). Thymus harvested from fetuses of co-vaccinated pregnant gilts had a lower number of PRRSV-1 and PRRSV-2 positive cells compared to the unvaccinated control group. Consequently, co-vaccinated pregnant gilts had a higher number of live-born piglets and decrease in the number of stillbirths.

We also measured T cell responses as the numbers of IFN-γ-SC. These cells play an important role in protective immunity because neutralizing antibody titers against PRRSV-1 and PRRSV-2 rarely reached above 1:32, which is the minimum neutralizing antibody titers to prevent abortion in pregnant sows under experimental conditions (Osorio et al., 2002). The activation of IFN-γ-SC correlated statistically with the time of reduction of PRRSV-1 and PRRSV-2 viremia (data not shown). These data provide scientific evidence that concurrent vaccination can elicit a protective cell-mediated immunity without interfering with one another.

The timing of the PRRSV challenge is also very critical in evaluating a PRRSV MLV vaccine because the virus has not yet crossed the placenta during early to mid-gestation (Christianson et al., 1993; Han et al., 2014). Therefore, late gestation around 93 days in the period is the most susceptible to congenital PRRSV infection (Karniychuk and Nauwynck, 2013). However, challenging with PRRSV at day 93 of gestation can complicate the timing of PRRSV vaccination because the duration of immunity (DOI) for PRRSV-1 and PRRSV-2 MLV vaccines is 16 and 19 weeks, respectively. Therefore, we adjusted the timing of vaccination to 3 weeks prior to breeding for both vaccines.

To our knowledge, this is the first experimental challenge study to evaluate the effect of concurrent vaccination against dual heterologous PRRSV-1 and PRRSV-2 challenge in terms of female reproductive

failure. Control of PRRSV-1 and PRRSV-2 by concurrent vaccination is clinically important because combined vaccine for PRRSV-1 and PRRSV-2 is not commercially available yet. PRRSV-1 and PRRSV-2 MLV vaccines are widely used for PRRSV control in sow populations within a farm. Our results, therefore, provide swine practitioners and producers with clinical data the beneficial effect of concurrent vaccination with PRRSV-1 and PRRSV-2 MLV vaccines.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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