



Comparison of two genetically distant type 2 porcine reproductive and respiratory syndrome virus (PRRSV) modified live vaccines against Vietnamese highly pathogenic PRRSV



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ABSTRACT

Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) known as pig high fever disease was first reported in China and has spread rapidly in neighboring southeastern Asian countries. The objective of this study was to evaluate the efficacy of a new type 2 PRRSV modified live vaccine (vaccine A) against a challenge with a HP-PRRSV and to compare the efficacy of two genetically distant type 2 PRRSV modified vaccines (vaccine A for lineage 8 and vaccine B for lineage 5) against HP-PRRSV (lineage 8) challenge. Pigs were divided into 4 groups ($n = 12/\text{group}$): vaccinated challenged (2 groups), unvaccinated challenged, and unvaccinated unchallenged groups. Regardless of vaccines, vaccinated challenged pigs showed significantly lower ($P < 0.05$) mean rectal temperatures and respiratory scores, levels of HP-PRRSV viremia, and lung lesions and HP-PRRSV antigens within lung lesions compared to unvaccinated challenged pigs. Vaccinated challenged pigs had significantly higher ($P < 0.05$) numbers of interferon- γ secreting cells (IFN- γ -SC) compared to unvaccinated challenged pigs. Significant differences were also found when comparing two type 2 PRRSV vaccines after HP-PRRSV challenge. The use of type 2 PRRSV vaccine A was able to significantly reduce fever when compared to type 2 PRRSV vaccine B in vaccinated challenged pigs. Vaccination of pigs with vaccine A reduced viral loads in their blood and induced higher numbers of HP-PRRSV-specific IFN- γ -SC than vaccination of pigs with vaccine B. This study demonstrates partial protection of two genetically distant type 2 PRRSV vaccines against HP-PRRSV challenge in growing pigs.

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped positive-strand RNA virus belonging to the order *Nidovirales*, family *Arteriviridae*, and genus *Arterivirus* with types 1 and 2 genotypes (Allende et al., 1999; Snijder and Meulenber, 1998). PRRSV is an important virus devastating to the swine industry worldwide (Snijder et al., 2013). In May 2006, an unusual large-scale outbreak initially called “pig high fever disease (PHFD)” severely affected the major pig-producing areas of Jiangxi Province of China and resulted in more than one million deaths in pigs (Tian et al., 2007). PHFD was initially classified as a hog cholera-like disease characterized by high fever (40–42 °C) and high mortality

(20–70%) in pigs of all ages (Tian et al., 2007; Li et al., 2007; Tong et al., 2007). The causative virus was identified and named as highly pathogenic PRRSV (HP-PRRSV) (Tian et al., 2007). HP-PRRSV is a variant of type 2 PRRSV containing a novel discontinuous 30-amino-acid (aa) deletion in nsp2 (Tian et al., 2007).

Since the first outbreak of HP-PRRSV infection in China in 2006, this disease spread subsequently to South East Asian countries including Vietnam and the Philippines in 2007, in Thailand in 2008, and Cambodia and Laos in 2010 (An et al., 2011; Ni et al., 2012; Tornimben et al., 2014; Jantafong et al., 2015). Vaccination is now considered to be one of the main strategies to control the HP-PRRSV. Three HP-PRRSV (JXA1-R, HuN4-F112, and TJM)-based modified live vaccines provide effective protection against HP-PRRSV and are available in China (Tian et al., 2009; Leng et al., 2012; Yu et al., 2015). However, these HP-PRRSV-based vaccines are limited for use in other Asian countries. Therefore, evaluation of commercial PRRSV vaccines used worldwide is necessary to control HP-PRRS infection in many Asian countries.

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Study of commercial type 2 PRRSV modified live vaccines (Ingelvac PRRS MLV, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO, USA) has been reported to be effective against HP-PRRSV (Wei et al., 2013; Lager et al., 2014). However, limited data are available for immunological (interferon- γ secreting cells), virological (levels of viremia), and pathological (lung lesion scores and PCV2 antigen-positive cells within the lung lesions) evaluation. In addition, a new type 2 PRRSV vaccine (Fostera PRRS, Zoetis, Florham Park, NJ, USA) has now entered Asian markets since 2013–2014. Interestingly, the vaccine virus from a new type 2 PRRSV vaccine belongs to the same lineage 8 as HP-PRRSV (Shi et al., 2010). Despite the fact that the degree of genetic homology between vaccine and challenge virus is not a good predict of vaccine efficacy (Prieto et al., 2008), the same lineage 8 of the vaccine virus and HP-PRRSV raises the possibility that a new vaccine may be efficacious in protecting pigs from HP-PRRSV challenge. However, whether there is protection from this new PRRSV vaccine against HP-PRRSV is not currently known. Moreover, to date, there have been no studies done to compare the two genetically distant type 2 PRRSV modified live vaccines (Fostera PRRS for lineage 8 and Ingelvac PRRS MLV for lineage 5) against HP-PRRSV.

The first objective of this study was to evaluate the efficacy of the recently introduced type 2 PRRSV vaccine (Fostera PRRS) against HP-PRRSV challenge in growing pigs. The second objective was to comprehensively compare the efficacy of two current type 2 PRRSV vaccines against HP-PRRSV challenge in growing pigs in terms of respiratory disease based on clinical, immunological, virological, and pathological outcomes.

2. Materials and methods

2.1. PRRSV inoculums

Vietnamese HP-PRRSV (strain MB6, Genbank number KM244760 and KM244761) was isolated from a 30-sow herd in a northern region of Vietnam in 2009 (Do et al., 2015). The strain MB6 caused the devastating respiratory disease with 30% mortality on the farm from which the virus originated. The selected Vietnamese HP-PRRSV is the cause of the recent Vietnamese outbreaks after the introduction to the northern areas and become the dominant strain (Dr. Do, personal observation). In previous experimental study, the most consistent lung lesion caused by Vietnamese HP-PRRSV strain MB6 was severe interstitial pneumonia with congestion or petechial hemorrhages on the surface of the lungs. The interstitial pneumonia was characterized by thickened alveolar septa with increased numbers of interstitial macrophages and lymphocytes and by type II pneumocyte hyperplasia (Do et al., 2015).

The Vietnamese HP-PRSV and vaccine virus (Foster PRRS classified in lineage 8, Zoetis) share 91.7% (93.2%) and 95.0% (91.7%) amino acids homology (nucleotides homology) for open reading frame (ORF) 5 and ORF7, respectively. The Vietnamese HP-PRRSV and vaccine virus (Ingelvac PRRS MLV classified lineage 5, Boehringer Ingelheim Vetmedica Inc.) shared 86.6% (86.9%) and 96.7% (93.4%) amino acids homology (nucleotides homology) for ORF5 and ORF7, respectively. The Vietnamese HP-PRRSV strain used on this study were analyzed phylogenetically together with Chinese HP-PRRSV strains and vaccine viruses listed in the GenBank data bases for ORF5 (Fig. 1).

2.2. Experimental design

A total of 48 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 (PCV2) and swine influenza virus according to routine serological testing. All piglets were negative for type 1 and type 2 PRRSV

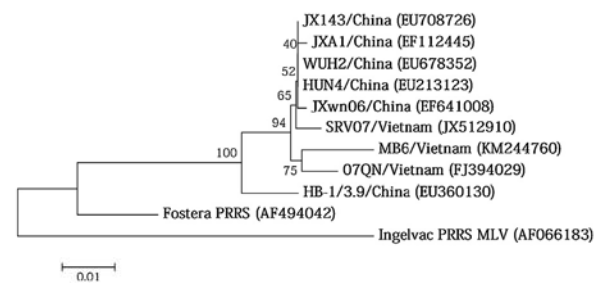


Fig. 1. Phylogenetic analysis of ORF5 from Vietnamese and Chinese HP-PRRSV, and vaccine viruses. An unrooted neighborjoining tree was constructed from aligned nucleic acid sequences.

viremia by real-time polymerase chain reaction (PCR) (Wasilk et al., 2004).

Pigs were divided into 4 groups ($n = 12/\text{group}$) using random number generation function (Excel, Microsoft Corporation, Redmond, WA, USA) (Table 1). The pigs in VacA/Ch were immunized with Fostera PRRS (Zoetis, Lot No. 1310547) administered as a 2.0 mL dose at 21 days of age based on the manufacturer's recommendations. The pigs in VacB/Ch were immunized with Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica Inc., Lot No. 245-F21) administered as a 2.0 mL dose at 21 days of age based on the manufacturer's recommendations. At 56 days of age (0 days post challenge, dpc), the pigs in VacA/Ch, VacB/Ch, and UnVac/Ch were inoculated intranasally with 3 mL of tissue culture fluid containing $10^{5.5}$ tissue culture infective dose 50% (TCID_{50})/mL of Vietnamese HP-PRRSV (strain MB6, 4th passage in MARC-145 cells) by setting them on their buttocks perpendicular to the floor and expending the neck fully back. The inoculum was slowly dripped into both nostrils of the pigs taking approximately 3–5 min/pig as previously described (Halbur et al., 1995). The pigs in UnVac/UnCh remained unvaccinated unchallenged and served as the negative control group. The pigs in each group were housed separately within the facility. Blood samples were collected at –35, –32, –28, –25, –21, –14, 0, 7, 10, and 14 dpc. Subsets of pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 7, 10, and 14 dpc as previously described (Beaver et al., 2001). Tissues were collected from each pig at necropsy. All of the methods were previously approved by the Nonglam University Institutional Animal Care and Use, and Ethics Committee (TN-TY 0012014.11.01).

2.3. Clinical observation

The pigs were monitored weekly for physical condition and scored daily for clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995). Rectal temperatures were recorded daily at the same time by the same personnel.

2.4. Quantification of PRRSV RNA

RNA was extracted from serum samples to quantify PRRSV genomic cDNA copy numbers, as previously described (Wasilk et al., 2004). Real-time PCR for HP-PRRSV were performed to quantify PRRSV genomic cDNA copy numbers (Do et al., 2015). Real-time PCR for the vaccine strains was also performed to quantify PRRSV genomic cDNA copy (Park et al., 2014; Han et al., 2014).

2.5. Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA;

Table 1

Clinical signs in pigs among 4 groups at different days post challenge (dpc).

	dpc	Groups			
		VacA/Ch	VacB/Ch	UnVac/Ch	UnVac/UnCh
Vaccines		Fosteria PRRS	Ingelvac PRRS MLV	None	None
Challenge		HP-PRRSV	HP-PRRSV	HP-PRRSV	None
Loss of appetite	3	0/12	0/12	0/12	0/12
	5	0/12	0/12	3/11	0/12
	7	1/12	2/12	5/11	0/12
	10	0/8	2/8	5/8	0/8
	14	0/4	0/4	2/4	0/4
Reluctant to move	3	0/12	0/12	0/12	0/12
	5	0/12	1/12	6/11	0/12
	7	2/12	5/12	10/11	0/12
	10	1/8	2/8	6/8	0/8
	14	0/4	2/4	3/4	0/4
Eye discharge/swollen	3	6/12	3/12	7/12	0/12
	5	5/12	4/12	8/11	0/12
	7	4/12	3/12	8/11	0/12
	10	2/8	2/8	5/8	0/8
	14	2/4	2/4	3/4	0/4
Erythema/cyanosis	3	0/12	0/12	0/12	0/12
	5	0/12	2/12	3/11	0/12
	7	0/12	2/12	5/11	0/12
	10	0/8	1/8	4/8	0/8
	14	0/4	0/4	2/4	0/4
Shivering	3	0/12	0/12	0/12	0/12
	5	0/12	0/12	0/11	0/12
	7	0/12	3/12	5/11	0/12
	10	0/8	2/8	4/8	0/8
	14	0/4	0/4	1/4	0/4

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 greater than 0.4, according to the manufacturer's instructions. Serum virus neutralization (SVN) tests were also performed with challenge HP-PRRSV (Yoon et al., 1994). Serum samples were considered to be positive for neutralizing antibodies (NAs) if the titer was greater than 2.0 ($\log_2$) (Zuckermann et al., 2007).

2.6. Enzyme-linked immunospot (ELISPOT) assay

The numbers of HP-PRRSV-specific IFN- γ secreting cells (IFN- γ -SC) were determined in peripheral blood mononuclear cells (PBMC) (Park et al., 2014; Meier et al., 2003; Diaz and Mateu, 2005).

2.7. Pathology and immunohistochemistry

Macroscopic and microscopic lung lesions were scored and analyzed morphometrically as previously described (Halbur et al., 1995). Tissue sections were blindly examined by two veterinary pathologists. Immunohistochemistry (IHC) for PRRSV was performed using SR30 monoclonal antibody (Rural Technologies Inc., Brookings, SD, USA) and analyzed morphometrically as previously described (Han et al., 2012; Halbur et al., 1996).

2.8. Virus isolation

Lungs were collected for virus isolation as previously described (Halbur et al., 1995).

2.9. Bacteria isolation

Lung tissues were collected and used for bacterial cultures on 5% bovine blood agar. Christie–Atkins–Munch–Petersen reaction was determined on 5% bovine blood agar, using a beta-hemolysis-producing strain of *Staphylococcus intermedius*.

2.10. Statistical analysis

Prior to statistical analysis, all real-time PCR and NAs 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, serology, IFN- γ -SC, and macroscopic lung lesion score) were analyzed using one-way ANOVA followed by Tukey's multiple comparison test at each time point. Discrete data (PRRSV antigen score, microscopic lung lesion score, and respiratory score) were analyzed by Mann–Whitney tests. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Clinical observation

Pigs in VacA/Ch showed significantly lower ($P < 0.05$) mean rectal temperatures than pigs in VacB/Ch from 5 to 10 dpc and pigs in UnVac/Ch from 2 and 13 dpc. Pigs in VacB/Ch showed significantly lower ($P < 0.05$) mean rectal temperature than pigs in UnVac/Ch at 5, 10, 12, and 13 dpc (Fig. 2A).

Clinical differences between vaccinated and unvaccinated challenged pigs are summarized in Table 1. Onset of disease in pigs in VacA/Ch and VacB/Ch was delayed several days and no mortality occurred during the experiment. Three pigs in UnVac/Ch died at 5 ($n=1$) and 10 ($n=2$) dpc. Intermittent erythema of the skin was present in 2 pigs from VacB/Ch and 3–5 pigs from UnVac/Ch beginning at 5 dpc. Several pigs in UnVac/Ch developed cyanotic extremities. Shivering was present in pigs in VacB/Ch beginning at 7 dpc and lasting until 10 dpc whereas shivering was also present in most pigs in UnVac/Ch beginning at 7 dpc but lasted until 14 dpc. Interestingly, the severity of clinical disease was less in pigs in VacA/Ch compared with pigs in VacB/Ch. Pigs in VacA/Ch did not show clinical signs such as cutaneous erythema and shivering. Pigs in VacA/Ch and VacB/Ch showed significantly lower ($P < 0.05$) mean respiratory scores than pigs in UnVac/UnCh at 2, 4, and 5 dpc (Fig. 2B). Negative control pigs maintained normal temperatures without respiratory symptoms throughout the experiment. No bacterial pathogens were isolated at necropsy.

3.2. Quantification of PRRSV RNA in sera

Genomic copies of the vaccine virus were detected in the serum collected from the vaccinated challenged pigs in both, VacA/Ch and VacB/Ch at –32 (3 days post vaccination), –30, –28, and –25 dpi but, thereafter, no genomic copies were detected in the sera of pigs from this group throughout the experiment. Genomic copies of HP-PRRSV were detected in the serum samples of pigs in VacA/Ch, VacB/Ch, and UnVac/Ch.

Pigs in VacA/Ch had a significantly lower ($P < 0.05$) number of genomic copies of HP-PRRSV in their blood compared to pigs in UnVac/Ch at 7 and 10 dpc. Pigs in VacA/Ch had a significantly lower ($P < 0.05$) number of genomic copies of HP-PRRSV in their blood compared with pigs in VacB/Ch at 10 dpc. Pigs in VacB/Ch had a significantly lower ($P < 0.05$) number of genomic copies of HP-PRRSV in their blood compared to pigs in UnVac/Ch at 5 and 7 dpc (Fig. 3). Prevalence rates of HP-PRRSV viremia are summarized in

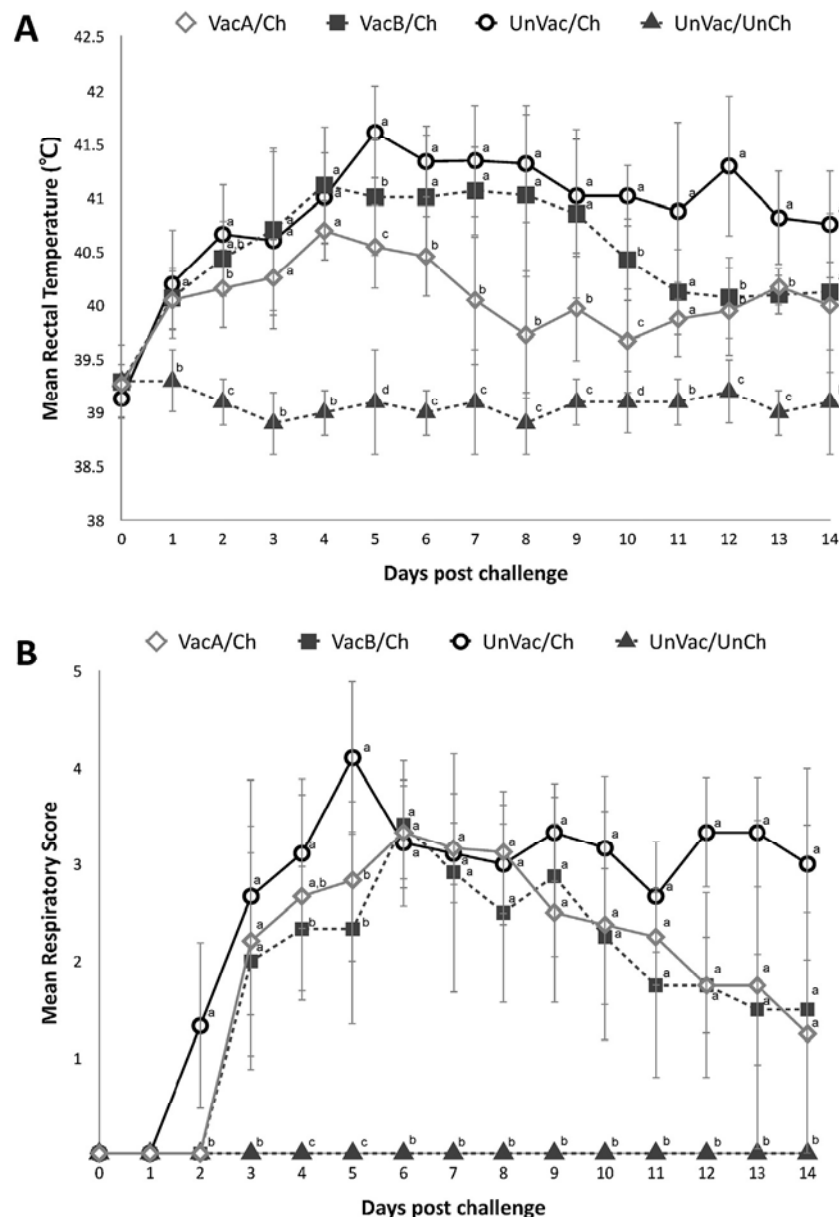


Fig. 2. Mean rectal temperatures (A) and mean respiratory score (B) from pigs in VacA/Ch (◇), VacB/Ch (■), UnVac/Ch (○), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Different letters (a, b, and c) indicate significant difference ($P < 0.05$) between groups.

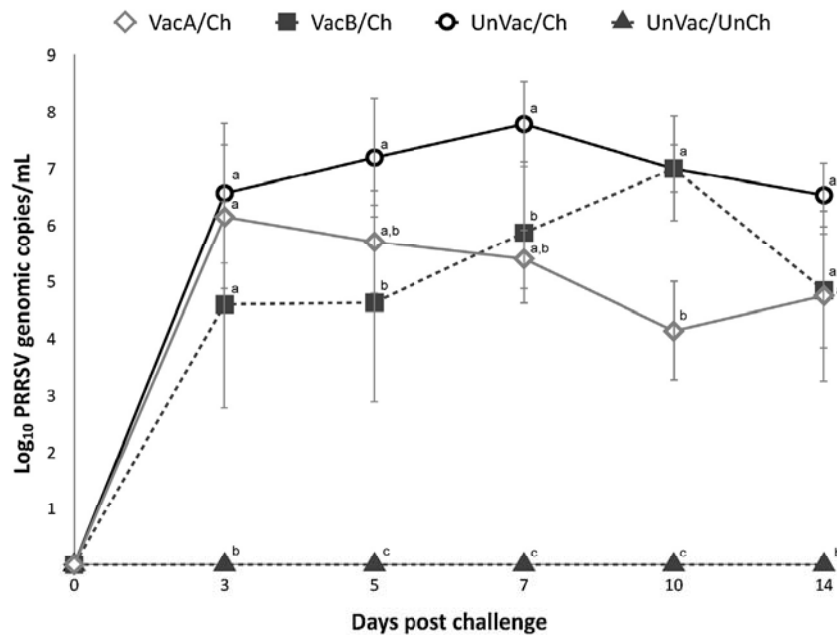


Fig. 3. Mean values of the genomic copy number of PRRSV RNA in serum in from pigs in VacA/Ch (◇), VacB/Ch (■), UnVac/Ch (○), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Different letters (a, b, and c) indicate significant difference ($P < 0.05$) between groups.

Table 2. No HP-PRRSV was detected in the sera of pigs from UnVac/UnCh throughout the experiment.

3.3. Anti-PRRSV antibodies

At the time of PRRSV vaccination (3 weeks of age; –35 dpc), pigs in 4 groups were seronegative. Antibodies specific for PRRSV were detected by ELISA in pigs in VacA/Ch and VacB/Ch from –14 to –7 dpc (10 days post vaccination) onward and in pigs in UnVac/Ch from 10 dpc onward. Pigs in VacA/Ch and VacB/Ch had significantly higher ($P < 0.05$) anti-PRRSV antibody titers than pigs in UnVac/Ch

from –25 to 10 dpc (Fig. 4). Anti-PRRSV antibody titers were not detected in negative control pigs at any time.

3.4. PRRSV-specific neutralizing antibodies

NAs were not detected in any pigs in any group (NAs titer $< 2 \log_2$) throughout the experiment.

3.5. Frequency of PRRSV-specific interferon- γ secreting cells

Upon challenge with HP-PRRSV, the frequency of HP-PRRSV-specific IFN- γ -SC increased gradually and reached an average of

Table 2
Proportion of viremic pigs, macroscopic and microscopic lung lesion score, and immunohistochemical antigen score of HP-PRRSV among 4 groups at different days post challenge (dpc).

	dpc	Groups			
		VacA/Ch	VacB/Ch	UnVac/Ch	UnVac/UnCh
Vaccines		Fostera PRRS	Ingelvac PRRS MLV	None	None
Challenge		HP-PRRSV	HP-PRRSV	HP-PRRSV	None
Proportion of viremic pigs	7	12/12	12/12	12/12	0/12
	10	8/8	8/8	8/8	0/8
	14	4/4	4/4	4/4	0/4
Macroscopic lung lesion score	7	82.8 $\pm$ 2.1 ^a	84.4 $\pm$ 2.8 ^a	90.8 $\pm$ 4.1 ^b	0/12
	10	70.0 $\pm$ 5.4 ^a	75.1 $\pm$ 4.4 ^{a,b}	84.4 $\pm$ 4.3 ^b	0/8
	14	50.0 $\pm$ 5.8 ^a	55 $\pm$ 5.4 ^a	75.6 $\pm$ 6.3 ^b	0/4
Microscopic lung lesion score	7	2.6 $\pm$ 0.07 ^a	2.6 $\pm$ 0.16 ^{a,b}	2.98 $\pm$ 0.20 ^b	0/12
	10	2.4 $\pm$ 0.2	2.46 $\pm$ 0.25	2.81 $\pm$ 0.25	0/8
	14	2.0 $\pm$ 0.4 ^a	2.29 $\pm$ 0.24 ^a	2.78 $\pm$ 0.09 ^b	0/4
PRRSV-antigen score	7	1.5 $\pm$ 0.4	1.65 $\pm$ 0.63	2.13 $\pm$ 0.24	0/12
	10	0.63 $\pm$ 0.75 ^a	1.67 $\pm$ 0.47 ^{a,b}	2.33 $\pm$ 0.47 ^b	0/8
	14	0.75 $\pm$ 0.5 ^a	0.88 $\pm$ 0.63 ^a	2.75 $\pm$ 0.65 ^b	0/4
Virus isolation	7	4/4	4/4	4/4	0/4
	10	3/4	4/4	4/4	0/4
	14	3/4	3/4	4/4	0/4

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

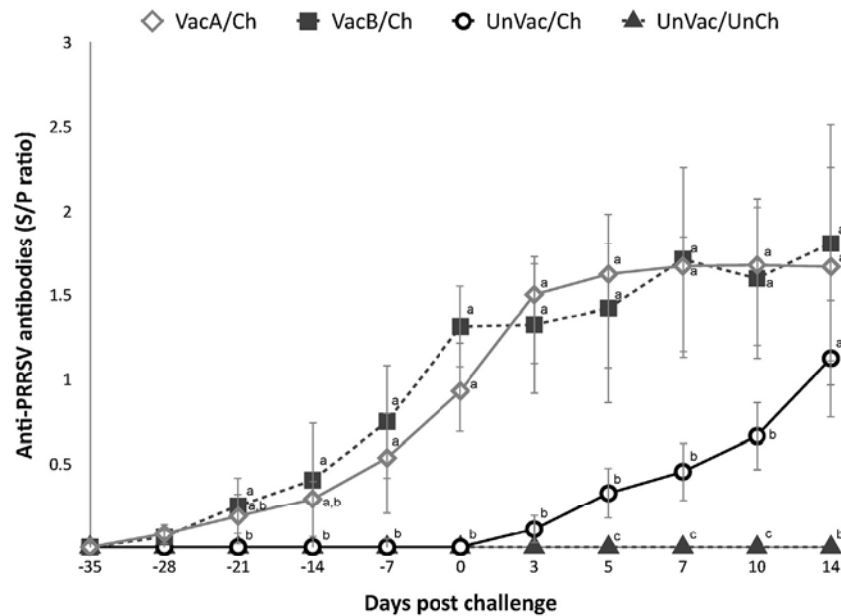


Fig. 4. Mean values of the anti-PRRSV antibodies from pigs in VacA/Ch (◇), VacB/Ch (■), UnVac/Ch (○), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Different letters (a, b, and c) indicate significant difference ($P < 0.05$) between groups.

207.0 ± 22.2 cells/ 10^6 PBMC (VacA/Ch) and 115.0 ± 26.5 cells/ 10^6 PBMC (VacB/Ch) at 14 dpc. Pigs in VacA/Ch produced significantly higher ($P < 0.05$) numbers of HP-PRRSV-specific IFN- γ -SC at 14 dpc compared to pigs in VacB/Ch. Pigs in VacA/Ch produced significantly higher ($P < 0.05$) numbers of HP-PRRSV-specific IFN- γ -SC at 10 and 14 dpc compared to pigs in UnVac/Ch group. Pigs in VacB/Ch produced significantly higher ($P < 0.05$) numbers of HP-PRRSV-specific IFN- γ -SC at 14 dpc compared to pigs in UnVac/Ch (Fig. 5). The mean frequencies of HP-PRRSV-specific IFN- γ -SC remained at basal levels (< 20 cells/ 10^6 PBMC) in the negative control pigs at any time.

3.6. Lung lesions

Macroscopic lung lesions were characterized by well-demarcated consolidated areas with diffuse tan-brown discoloration in the middle, caudal and accessory lobes. Pigs in VacA/Ch and VacB/Ch had significantly fewer ($P < 0.05$) mean macroscopic lung lesion scores than pigs in UnVac/Ch at 7, 10, and 14 dpc (Table 2). Microscopic lung lesions were characterized by alveolar septa thickened with interstitial infiltrates of macrophages and lymphocytes, by type II pneumocyte hyperplasia, and by accumulation of normal and necrotic macrophages in alveolar spaces. Pigs in VacA/Ch had significantly lower ($P < 0.05$) mean microscopic lung lesion scores than pigs in UnVac/Ch at 7 and 14 dpc. Pigs in VacB/Ch

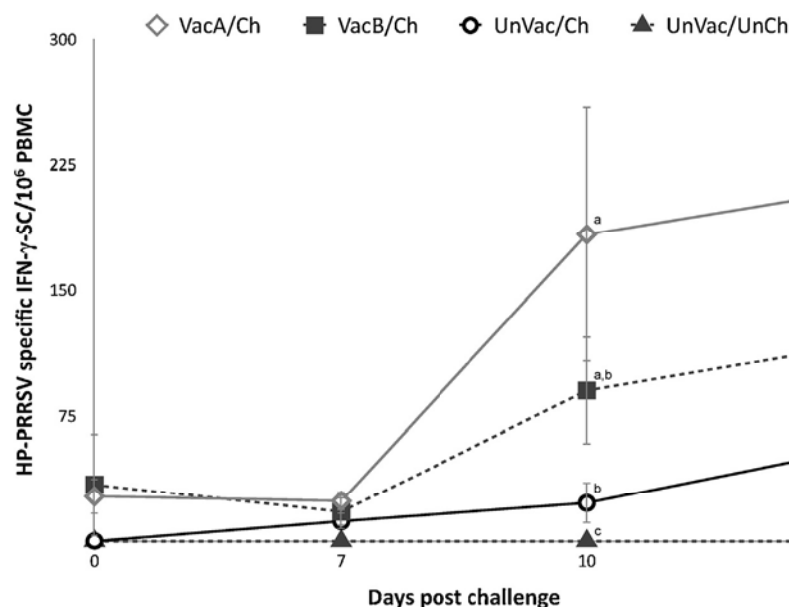


Fig. 5. Frequency of challenging HP-PRRSV-specific IFN- γ -SC/ 10^6 PBMC from pigs in VacA/Ch (◇), VacB/Ch (■), UnVac/Ch (○), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Different letters (a, b, c, and d) indicate significant difference ($P < 0.05$) between groups.

had significantly lower ($P < 0.05$) mean microscopic lung lesion scores than pigs in UnVac/Ch at 14 dpc (Table 2). No macroscopic and microscopic lung lesions were observed in negative control pigs throughout the experiment.

3.7. Immunohistochemistry

PRRSV antigens were detected exclusively within the cytoplasm of macrophages and pneumocytes. Positive cells typically exhibited a red reaction product by immunohistochemistry. The score for the mean number of PRRSV-positive cells per unit area of lung was significantly fewer ($P < 0.05$) in pigs in VacA/Ch than in pigs in UnVac/Ch at 10 and 14 dpc. The score for the mean number of PRRSV-positive cells per unit area of lung was significantly fewer ($P < 0.05$) in pigs in VacB/Ch than in pigs in UnVac/Ch at 14 dpc (Table 2, Fig. 6). No immunohistochemical staining for PRRSV

antigen was observed in lung sections from the negative control pigs.

3.8. Virus isolation

Virus isolation from lungs is summarized in Table 2. The isolated HP-PRRSV was confirmed to be the same as the challenge virus through sequence analysis. No PRRSV was isolated from negative control pigs.

4. Discussion

The results of the present study demonstrate that two commercial type 2 PRRSV vaccines are efficacious in protecting growing pigs against HP-PRRSV challenge. Generally, PRRSV modified live vaccines offer protection against homologous strains but are inconsistent and offer incomplete protection against heterologous strains (Lager et al., 1997, 1999; Mengeling et al., 1999, 2003). HP-PRRSV (JXA1-R, HuN4-F112, and TJM)-derived modified live vaccines provide effective homologous protection against the same HP-PRRSV strains challenge (Tian et al., 2009; Leng et al., 2012; Yu et al., 2015). However, these HP-PRRSV-based vaccines are limited for use in other Asian countries. Therefore, evaluation of commercial PRRSV vaccines used worldwide is necessary in order to control HP-PRRS infection in many Asian countries. The same commercial type 2 PRRSV vaccine (Ingelvac PRRS MLV) used in this study also protects pigs against Chinese HP-PRRSV and infectious clone of Vietnamese HP-PRRSV challenge (Wei et al., 2013; Lager et al., 2014). In contrast, commercial type 1 PRRSV vaccine offered a limited protection against Vietnamese HP-PRRSV challenge (Roca et al., 2012). These results indicate that type 2 PRRSV vaccine is more efficacious in control of HP-PRRSV infection in pigs compared to type 1 PRRSV vaccine.

Clinical analysis is a critical parameter to evaluate the efficacy of the PRRSV vaccines against HP-PRRSV because the two most clinical characteristics of HP-PRRS infection are high fever ($41\text{--}42^\circ\text{C}$) and high mortality (20–70%) (Tian et al., 2007; Li et al., 2007, 2012; Tong et al., 2007; Hu et al., 2013; Wang et al., 2014). Although the use of PRRSV vaccines reduced body temperatures and mortality in this study, there were differences in the reduction of body temperature between the two commercial type 2 PRRSV vaccines. The use of type 2 PRRSV vaccine A was able to significantly reduce fever when compared to type 2 PRRSV vaccine B.

Severity of a respiratory disease is well correlated with the amount of viral load (Johnson et al., 2004). Therefore, measurement of viral loads in blood and lungs is important to determine the efficacy of PRRSV vaccine. Both commercial type 2 PRRSV vaccines are able to reduce the levels of HP-PRRSV viremia and antigen within lung lesions although there were statistical significant differences in viral reduction between two type 2 PRRSV vaccines. The mechanisms of the partial protection including clinical signs and viremic reduction shown in the study may be due to induction of IFN- γ -SC. In the present study, type 2 PRRSV vaccine A induced higher numbers of HP-PRRSV-specific IFN- γ -SC compared to type 2 PRRSV vaccine B. These differences may result in significant differences in viremic reduction between two type 2 PRRSV vaccines after HP-PRRSV challenge. Similarly, the reduction of HP-PRRSV viremia coincides with the appearance of HP-PRRSV-specific IFN- γ -SC in vaccinated challenged pigs similar to a previous study (Park et al., 2014). Moreover, correlation is also observed between serum viral load and IFN- γ levels in the sera (Loving et al., 2008). These results suggest that numbers of HP-PRRSV-specific IFN- γ -SC induced by two type 2 PRRSV vaccines may be sufficient enough to clear the challenged HP-PRRSV from pigs.

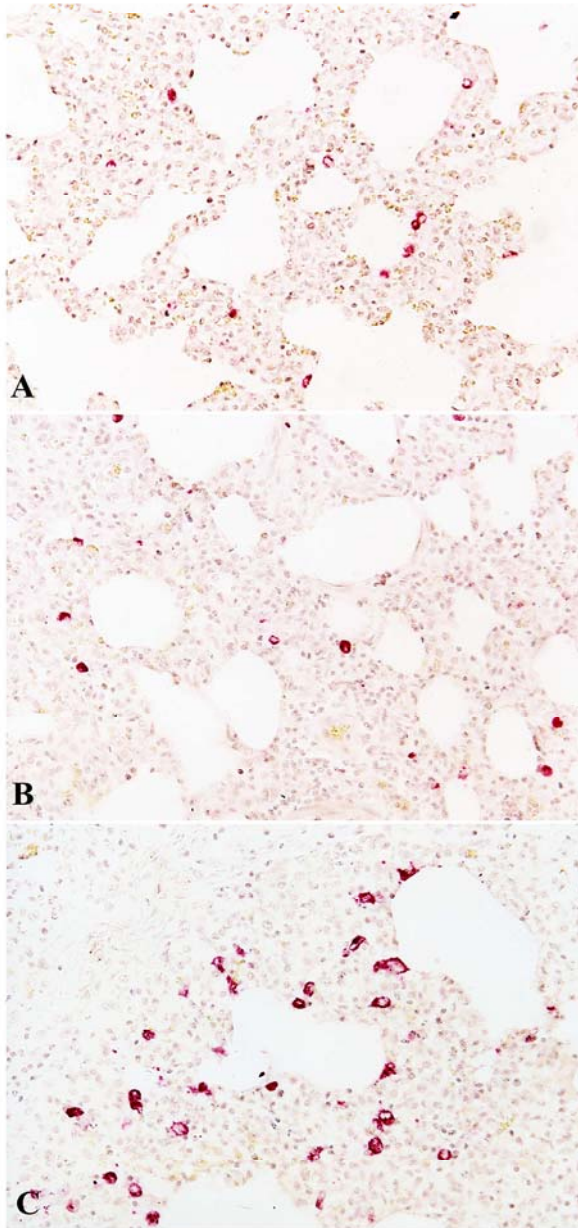


Fig. 6. Immunohistochemistry of PRRSV antigen. A few PRRSV antigens were detected in lungs with mild interstitial pneumonia from pigs in VacA/Ch (A) and VacB/Ch (B), and numerous PRRSV antigens were detected in lungs with severe interstitial pneumonia from pigs in UnVac/Ch (C) at 10 days post challenge.

The prediction of protection has frequently been attributed to antigenic but not genetic similarity between vaccine and challenge strains (Lager et al., 1999; Prieto et al., 2008). In the present study, type 2 PRRSV vaccine A provided slightly better protection against HP-PRRSV of the same genomic lineage compared to type 2 PRRSV vaccine B which is of a different genomic lineage based on body temperature, levels of viremia, and number of IFN- γ -SC. However, the results of our study should be interpreted carefully. The genetic similarity between vaccine and field virus does not guarantee vaccine efficacy (Prieto et al., 2008). These differences between type 2 PRRSV vaccine A and B may suggest that type 2 PRRSV vaccine A virus and challenge HP-PRRSV that are closely related genetically may be also closely related antigenically.

To the best of our knowledge, this is the first evaluation of a new type 2 PRRSV vaccine against HP-PRRSV challenge and a comprehensive comparison of two commercial type 2 PRRSV vaccines against HP-PRRSV challenge based on clinical, virological, immunological, and pathological analysis under experimental conditions. HP-PRRSV is now a severe threat to the whole Southeast Asian pig population and could further spread to other continents. Geographical proximity especially generates great concerns about its potential spread to neighboring countries such as Korea and Japan in the future. Regardless of commercial vaccines, vaccination of pigs with type 2 PRRSV is able to reduce clinical signs, the levels of HP-PRRSV viremia, and the severity of lung lesions after the Vietnamese HP-PRRSV challenge.

5. Conclusions

Two genetically distant type 2 PRRSV modified live vaccines provide partial protection against respiratory disease in growing pigs against HP-PRRSV challenge. Regardless of vaccines, vaccinated challenged pigs showed significantly lower ($P < 0.05$) mean rectal temperatures and respiratory scores, levels of HP-PRRSV viremia, lung lesions and HP-PRRSV antigens within lung lesions compared to unvaccinated challenged pigs.

Competing interests

None.

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References

- Allende, R., Lewis, T.L., Lu, Z., Rock, D.L., Kutish, G.F., Ali, A., Oster, A.R., Osorio, F.A., 1999. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. *J. Gen. Virol.* 80, 307–315.
- An, T.-Q., Tian, Z.-J., Leng, C.-L., Peno, J.-M., Tong, G.-Z., 2011. Highly pathogenic porcine reproductive and respiratory syndrome virus, Asia. *Emerg. Infect. Dis.* 17, 1782–1784.
- Beaver, B.V., Reed, W., Leary, S., McKiernan, B., Bain, F., Schultz, R., Bennett, B.T., Pascoe, P., Shull, E., Cork, L.C., Francis-Floyd, R., Amass, K.D., Johnson, R., Schmidt, R.H., Underwood, W., Thornton, G.W., Kohn, B., 2001. 2002 Report of the AVMA panel on euthanasia. *J. Am. Vet. Med. Assoc.* 218, 669–696.
- Diaz, I., Mateu, E., 2005. Use of ELISPOT and ELISA to evaluate IFN- γ , IL-10 and IL-4 responses in conventional pigs. *Vet. Immunol. Immunopathol.* 106, 107–112.
- Do, T.D., Park, C., Choi, K., Jeong, J., Nguyen, T.T., Nguyen, D.Q., Le, T.H., Chae, C., 2015. Comparison of northern and southern Vietnamese highly pathogenic porcine reproductive and respiratory syndrome virus in experimentally infected pigs. *J. Comp. Pathol.* 152, 227–237.
- Halbur, P.G., Paul, P.S., Frey, M.L., Landgraf, J., Eernisse, K., Meng, X.-J., Lum, M.A., Andrews, J.J., Rathje, J.A., 1995. Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. *Vet. Pathol.* 32, 648–660.
- Halbur, P.G., Paul, P.S., Frey, M.L., Landgraf, J., Eernisse, K., Meng, X.-J., Andrews, J.J., Lum, M.A., Rathje, J.A., 1996. Comparison of the antigen distribution of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. *Vet. Pathol.* 33, 159–170.
- Han, K., Seo, H.W., Oh, Y., Kang, I., Park, C., Kang, S.H., Kim, S.-H., Lee, B.-H., Kwon, B., Chae, C., 2012. Evaluation of monoclonal antibody-based immunohistochemistry for the detection of European and North American porcine reproductive and respiratory syndrome virus and a comparison with in situ hybridization and reverse transcription polymerase chain reaction. *J. Vet. Diagn. Invest.* 24, 719–724.
- Han, K., Seo, H.W., Park, C., Chae, C., 2014. Vaccination of sows against type 2 porcine reproductive and respiratory syndrome virus (PRRSV) before artificial insemination protects against type 2 PRRSV challenge but does not protect against type 1 PRRSV challenge in late gestation. *Vet. Res.* 45, 12.
- Hu, S.P., Zhang, Z., Liu, Y.G., Tian, Z.J., Wu, D.L., Cai, X.H., He, X.J., 2013. Pathogenicity and distribution of highly pathogenic porcine reproductive and respiratory syndrome virus in pigs. *Transbound. Emerg. Dis.* 60, 351–359.
- Jantafong, T., Sangtong, P., Saengub, W., Mungkundar, C., Romlamduan, N., Lekchareonsuk, C., Lekchareonsuk, P., 2015. Genetic diversity of porcine reproductive and respiratory syndrome virus in Thailand and Southeast Asia from 2008 to 2013. *Vet. Microbiol.* 176, 229–238.
- Johnson, W., Roof, M., Vaughn, E., Christopher-Hennings, J., Johnson, C.R., Murtaugh, M.P., 2004. Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection. *Vet. Immunol. Immunopathol.* 102, 233–247.
- Lager, K.M., Mengeling, W.L., Brockmeier, S.L., 1997. Duration of homologous porcine reproductive and respiratory syndrome virus immunity in pregnant swine. *Vet. Microbiol.* 58, 127–133.
- Lager, K.M., Mengeling, W.L., Brockmeier, S.L., 1999. Evaluation of protective immunity in gilts inoculated with the NADC-8 isolate of porcine reproductive and respiratory syndrome virus (PRRSV) and challenge-exposed with an antigenically distinct PRRSV isolate. *Am. J. Vet. Res.* 60, 1022–1027.
- Lager, K.M., Schlink, S.N., Brockmeier, S.L., Miller, L.C., Henningson, J.N., Kappes, M.A., Kehrl, M.E., Loving, C.L., Guo, B., Swenson, S.L., Yang, H.-C., Faaborg, K.S., 2014. Efficacy of type 2 PRRSV vaccine against Chinese and Vietnamese HP-PRRSV challenge in pigs. *Vaccine* 32, 6457–6462.
- Leng, X., Li, Z., Xia, M., He, Y., Wu, H., 2012. Evaluation of the efficacy of an attenuated live vaccine against highly pathogenic porcine reproductive and respiratory syndrome virus in young pigs. *Clin. Vaccine Immunol.* 19, 1199–1206.
- Li, Y., Wang, X., Bo, K., Wang, X., Tang, B., Yang, B., Jiang, W., Jiang, P., 2007. Emergence of a highly pathogenic porcine reproductive and respiratory syndrome virus in the Mid-Eastern region of China. *Vet. J.* 174, 577–584.
- Li, L., Zhao, Q., Ge, X., Teng, K., Kuang, Y., Chen, Y., Guo, X., Yang, H., 2012. Chinese highly pathogenic porcine reproductive and respiratory syndrome virus exhibits more extensive tissue tropism for pigs. *Vet. J.* 9, 1–6.
- Loving, C.L., Brockmeier, S.L., Vincent, A.L., Lager, K.M., Sacco, R.E., 2008. Differences in clinical disease and immune response of pigs challenged with a high-dose versus low-dose inoculums of porcine reproductive and respiratory syndrome virus. *Viral Immunol.* 21, 315–326.
- Meier, W.A., Galeota, J., Osorio, F.A., Husmann, R.J., Schnitzlein, W.M., Zuckermann, F.A., 2003. Gradual development of the interferon-gamma response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. *Virology* 309, 18–31.
- Mengeling, W.L., Lager, K.M., Vorwald, A.C., 1999. Safety and efficacy of vaccination of pregnant gilts against porcine reproductive and respiratory syndrome. *Am. J. Vet. Res.* 60, 796–801.
- Mengeling, W.L., Lager, K.M., Vorwald, A.C., Clouser, D.F., 2003. Comparative safety and efficacy of attenuated single-strain and multi-strain vaccines for porcine reproductive and respiratory syndrome. *Vet. Microbiol.* 93, 25–38.
- Ni, J., Yang, S., Bounlom, D., Yu, X., Zhou, Z., Song, J., Khamphouth, V., Vathana, T., Tian, K., 2012. Emergency and pathogenicity of highly pathogenic porcine reproductive and respiratory syndrome virus in Vientiane, Lao People's Democratic Republic. *J. Vet. Diagn. Invest.* 24, 349–354.
- Park, C., Seo, H.W., Han, K., Kang, I., Chae, C., 2014. Evaluation of the efficacy of a new modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine (Fostera PRRS) against heterologous PRRSV challenge. *Vet. Microbiol.* 172, 432–442.
- Prieto, C., Alvarez, E., Martinez-Lobo, F.J., Simarro, I., Castro, J.M., 2008. Similarity of European porcine reproductive and respiratory syndrome virus strains to vaccine strain is not necessarily predictive of the degree of protective immunity conferred. *Vet. J.* 175, 356–363.
- Roca, M., Gimeno, M., Bruguera, S., Segalés, J., Díaz, I., Galindo-Cardiel, I.J., Martínez, E., Darwich, L., Fang, Y., Maldonado, J., March, R., Mateu, E., 2012. Effect of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. *Vet. J.* 193, 92–96.
- Shi, M., Lam, T.T.-Y., Hon, C.-C., Murtaugh, M.P., Davies, P.R., Hui, R.K.-H., Li, J., Wong, L.T.-W., Yip, C.-W., Jiang, J.-W., Leung, F.C.-C., 2010. Phylogeny-based evolutionary, demographical, and geographical dissection of North American type 2 porcine reproductive and respiratory syndrome viruses. *J. Virol.* 84, 8700–8711.

- Snijder, E.J., Meulenbergh, J.J.M., 1998. The molecular biology of arteriviruses. *J. Gen. Virol.* 79, 961–979.
- Snijder, E.J., Kikkert, M., Fang, Y., 2013. Arterivirus molecular biology and pathogenesis. *J. Gen. Virol.* 94, 2141–2163.
- Tian, K., Yu, X., Zhao, T., Feng, Y., Cao, Z., Wang, C., Hu, Y., Chen, X., Hu, D., Tian, X., Liu, D., Zhang, S., Deng, X., Ding, Y., Yang, L., Zhang, Y., Xiao, H., Qiao, M., Wang, B., Hou, L., Wang, X., Yang, X., Kang, L., Sun, M., Jin, P., Wang, S., Kitamura, Y., Yan, J., Gao, G.F., 2007. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. *Plos One* 2, e526.
- Tian, Z.-J., An, T.-Q., Zhou, Y.-J., Peng, J.-M., Hu, S.-P., Wei, T.-C., Jiang, Y.-F., Xiao, Y., Tong, G.-Z., 2009. An attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) protects piglets against HP-PRRS. *Vet. Microbiol.* 138, 34–40.
- Tong, G.-Z., Zhou, Y.-J., Hao, X.-F., Tian, Z.-J., An, T.-Q., Qiu, H.-J., 2007. Highly pathogenic porcine reproductive and respiratory syndrome, China. *Emerg. Infect. Dis.* 13, 1434–1436.
- Tornimbene, B., Frossard, J.-P., Chhim, V., Sorn, S., Guitian, J., Drew, T.W., 2014. Emergence of highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) in medium-scale swine farms in southeastern Cambodia. *Prev. Vet. Med.* 118, 93–103.
- Wang, G., He, Y., Tu, Y., Liu, Y., Zhou, E.-M., Han, Z., Jiang, C., Wang, S., Shi, W., Cai, X., 2014. Comparative analysis of apoptotic changes in peripheral immune organs and lungs following experimental infection of piglets with highly pathogenic and classical porcine reproductive and respiratory syndrome virus. *Virol. J.* 11, 1–5.
- Wasilk, A., Callahan, J.D., Christopher-Hennings, J., Gay, T.A., Fang, Y., Dammen, M., Reos, M.E., Torremorell, M., Polson, D., Mellencamp, M., Nelson, E., Nelson, W.M., 2004. Detection of U.S., Lelystad, and European-like porcine reproductive and respiratory syndrome viruses and relative quantitation in boar semen and serum samples by real-time PCR. *J. Clin. Microbiol.* 42, 4453–4461.
- Wei, Z., Zhang, J., Zhuang, J., Sun, Z., Gao, F., Yuan, S., 2013. Immunization of pigs with a type 2 modified live PRRSV vaccine prevents the development of a deadly long lasting hyperpyrexia in a challenge study with highly pathogenic PRRSV JX143. *Vaccine* 31, 2062–2066.
- Yoon, I.J., Joo, H.S., Goyal, S.M., Molitor, T.W., 1994. A modified serum neutralization test for the detection of antibody to porcine reproductive and respiratory syndrome virus in swine sera. *J. Vet. Diagn. Invest.* 6, 289–292.
- Yu, X., Zhou, Z., Cao, Z., Wu, J., Zhang, Z., Xu, B., Hui, D., Deng, X., Han, W., Gu, X., Zhang, S., Li, X., Wang, B., Zhai, X., Tian, K., 2015. Assessment of the safety and efficacy of an attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV). *Clin. Vaccine Immunol.* 22, 493–502.
- Zuckermann, F.A., Garcia, E.A., Luque, I.D., Christopher-Hennings, J., Doster, A., Brito, M., Osorio, F., 2007. Assessment of the efficacy of commercial porcine reproductive and respiratory syndrome virus (PRRSV) vaccines based on measurement of serologic response, frequency of gamma-IFN-producing cells and virological parameters of protection upon challenge. *Vet. Microbiol.* 123, 69–85.