



Evaluation of a 20 year old porcine reproductive and respiratory syndrome (PRRS) modified live vaccine (Ingelvac[®] PRRS MLV) against two recent type 2 PRRS virus isolates in South Korea



Jiwoon Jeong¹, Kyuhyung Choi¹, Ikjae Kang, Changhoon Park, Chanhee Chae*

Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea

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ABSTRACT

Type 2 porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) was first isolated in Korea in 1994. The commercial PRRS modified live vaccine (Ingelvac[®] PRRS MLV, Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA) based on type 2 PRRSV, was first licensed for use in 3- to 18-week-old pigs in Korea in 1996. The objective of the present study was to evaluate the efficacy of this 20 year old commercial PRRS modified live vaccine (MLV) against two recent PRRSV isolates. Two genetically distant type 2 PRRSV strains (SNUVR150004 for lineage 1 and SNUVR150324 for lineage 5), isolated in 2015, were used as challenge virus. Regardless of the challenge virus, vaccination of pigs effectively reduced the level of viremia, the lung lesions, and of the PRRSV antigen within the lung lesions. The induction of virus-specific interferon- γ secreting cells by the PRRS vaccine produced a protective immune response, leading to the reduction of PRRSV viremia. There were no significant differences in efficacy against the two recently isolated viruses by the PRRS MLV based on virological results, immunological responses, and pathological outcomes. This study demonstrates that the PRRS MLV used in this study is still effective against recently isolated heterologous type 2 PRRSV strains even after 20 years of use in over 35 million pigs

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) has been one of the most economically important global viral diseases for over two decades. PRRS is characterized by reproductive failures in sows and respiratory distress in growing pigs (Zimmerman et al., 2012). The etiologic agent of PRRS is the PRRS virus (PRRSV), which is a member of the *Arterivirus* genus, *Arteriviridae* family and *Nidovirales* order (Snijder and Meulenber, 1998). The PRRSV genome is approximately 15 kb in length and contains at least ten open reading frames (ORFs) (Snijder et al., 2013). PRRSV can be divided into two genetically distinct genotypes: type 1 PRRSV, which is the major genotype circulating in Europe, and type 2 PRRSV, which is the major genotype found in North America and Asian countries (Allende et al., 1999; Murtaugh et al., 2010). Type 2 PRRSV is the most dominant and economically significant

genotype in Korea. Type 2 PRRSV is further classified into 9 lineages based on global genotyping classification (Shi et al., 2010). Among those, type 2 PRRSV belonging to lineage 1 and 5 is commonly isolated in Korea (Shi et al., 2010).

PRRSV is considered one of the most rapidly evolving viruses on the planet (Normile, 2007). Mutation and recombination are two common evolutionary mechanisms for PRRSV, which can lead to enhanced fitness for survival or increased virulence (Gorbalenya et al., 2006; Domingo and Holland, 1997). Rapid evolution of PRRSV is an important driving force for the emergence of new strains capable of vaccine resistance (Chand et al., 2012). The commercial PRRS modified live vaccine (Ingelvac[®] PRRS MLV, Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA) has been used to control PRRSV for 20 years in Korean pig farms. After 20 years of use, some swine producers and practitioners have raised the concerns about the efficacy of this PRRS MLV due to genetic and antigenic change of field viruses. Therefore, the objective of this study was to evaluate this PRRS MLV against two recent PRRSV isolates.

* Corresponding author.

E-mail address: swine@snu.ac.kr (C. Chae).

¹ Both authors contributed equally to this work.

2. Materials and methods

2.1. PRRSV inoculum

Type 2 PRRSV (SNUVR150004 strain, lineage 1, GenBank no. KU301047) was isolated from lung samples of growing pigs at 84 days of age in a 500-sow herd in 2015. This herd showed severe respiratory problems in growing pigs aged 10–18 weeks old. Another type 2 PRRSV (SNUVR150324 strain, lineage 5, GenBank no. KU301048) was isolated from lung samples of weaned pigs at 42 days of age in a 1000-sow herd in 2015. This herd had suffered recent losses due to type 2 PRRSV infection and respiratory diseases in weaned pigs.

2.2. Experimental design

A total of 130 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 *Mycoplasma hyopneumoniae* according to routine serological testing. Serum samples were negative for PCV2 and PRRSV, and nasal swabs were negative for *M. hyopneumoniae* when tested by real-time polymerase chain reaction (PCR) (Dubosson et al., 2004; Wasilk et al., 2004; Gagnon et al., 2008).

The pigs were randomly divided into 5 groups: Vac/Ch2L1 ($n=30$), Vac/Ch2L5 ($n=30$), UnVac/Ch2L1 ($n=30$), UnVac/Ch2L5 ($n=30$), and UnVac/UnCh ($n=10$) (Table 1). The pigs in Vac/Ch2L1 and Vac/Ch2L5 were vaccinated with Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica; Lot No. 2451017A) and challenged with type 2 PRRSV lineage 1 and 5, respectively. A dose of 2 mL of Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica) was delivered by intramuscular injection on the right side of the neck at 21 days of age, according to the manufacturer's instructions.

Type 2 PRRSV inoculum consisted of either SNUVR150004 strain or SNUVR150324 strain, which was propagated on MARC-145 cells to a titer of 10^5 50% tissue culture infective doses (TCID₅₀)/mL. At 56 days of age (0 day post challenge, dpc), the pigs in Vac/Ch2L1 and UnVac/Ch2L1 were inoculated intranasally with 3 mL of type 2 PRRSV (SNUVR150004 strain, lineage 1) inoculums by setting them on their buttocks perpendicular to the floor and extending 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 Vac/Ch2L5 and UnVac/Ch2L5 were inoculated intranasally with 3 mL of type 2 PRRSV (SNUVR150324 strain, lineage 5) inoculums by the same

manner. The pigs in UnVac/UnCh served as negative controls and were neither vaccinated nor challenged. Fifteen and five pigs from each treatment (Vac/Ch2L1, Vac/Ch2L5, UnVac/Ch2L1, and UnVac/Ch2L5) and negative control (UnVac/UnCh) group, respectively, were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 7 and 14 dpc as previously described (Beaver et al., 2001).

The pigs in each group were housed in separate experimental rooms equipped with air conditioning and high-efficiency particulate air filtration to avoid possible transmission of the pathogen between groups throughout the experiment in the research facility. Following PRRSV inoculation, the physical condition of the pigs was monitored daily including rectal temperatures. Blood samples were collected at –35, –28, –21, –14, 0, 3, 7, 10, and 14 dpc. All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee.

2.3. Clinical observation

Following vaccination and PRRSV challenge, the pigs were monitored weekly for physical conditions 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). Observers were blinded to vaccination status. Stress was induced daily by pig handler by holding the pig under his arm and taking the rectal temperature (Halbur et al., 1996b). Rectal thermometer (Digital Fever Thermometer, Becton-Dickinson, Franklin Lakes, New Jersey, USA) was lubricated and inserted approximately 6–7 cm into the rectum and readings were taken when the thermometer beeped (Thoresen et al., 2001). Rectal temperatures were recorded daily at the same time by 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). For the challenge type 2 PRRSV, the forward and reverse primers were 5'-TGGCCAGTCAGTCAATCAAC-3' and 5'-AATCGATTGCAAGCAGAGGGAA-3', respectively (Park et al., 2014). For the vaccine virus, the forward and reverse primers were 5'-CTAACAAATTTGATTGGGCAG-3' and 5'-AGGACATGCAATCTTTGCAA-3', respectively (Han et al., 2011). Real-time PCR for the

Table 1

Experimental design and results of lesion score and porcine reproductive and respiratory syndrome virus (PRRSV) RNA within lung lesion at 7 and 14 days post challenge (dpc).

Groups	PRRSV		dpc (n)	Lung lesion score		PRRSV-positive cells within lung lesion
	Vaccination (21 days)	Challenge (56 days)		Macroscopic	Microscopic	
Vac/Ch2L1	Yes	Lineage 1	7 (15)	31.06 ± 6.40 ^a	1.93 ± 0.25 ^a	30.33 ± 5.66 ^a
			14 (15)	20.46 ± 4.41 ^a	1.27 ± 0.44 ^a	21.27 ± 4.33 ^a
Vac/Ch2L5	Yes	Lineage 5	7 (15)	32.09 ± 6.80 ^a	1.8 ± 0.4 ^a	28.4 ± 5.21 ^a
			14 (15)	21.73 ± 5.66 ^a	1.2 ± 0.4 ^a	21.07 ± 3.60 ^a
UnVac/Ch2L1	No	Lineage 1	7 (15)	56.13 ± 8.17 ^b	3.67 ± 0.47 ^b	42.6 ± 4.64 ^b
			14 (15)	38.25 ± 5.79 ^b	3.27 ± 0.57 ^b	34.13 ± 4.36 ^b
UnVac/Ch2L5	No	Lineage 5	7 (15)	61.33 ± 8.16 ^b	3.67 ± 0.60 ^b	42.36 ± 5.84 ^b
			14 (15)	38.48 ± 6.24 ^b	3 ± 0.37 ^b	34.81 ± 3.96 ^b
UnVac/UnCh	No	No	7 (5)	4.44 ± 2.64 ^c	0.2 ± 0.4 ^c	0 ^c
			14 (5)	4.76 ± 3.56 ^c	0.4 ± 0.49 ^c	0 ^c

^an = Numbers of pigs were necropsied at 7 and 14 dpc. Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.

challenge and vaccine viruses was performed as previously described (Han et al., 2011; Park et al., 2014).

2.5. Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS 3XR™, IDEXX Laboratories Inc., Westbrook, Maine, 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 the vaccine and the challenging viruses, as previously described (Yoon et al., 1994). The neutralizing antibodies (NAs) titers of each serum were determined as the reciprocal of the highest dilution in which no evidence of virus growth was detected. Serum samples were considered to be positive for 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 PRRSV-specific interferon- γ secreting cells (IFN- γ -SC) were determined in peripheral blood mononuclear cells (PBMC) as previously described (Meier et al., 2003; Diaz et al., 2005; Park et al., 2014) with some modifications. Briefly, 5×10^5 PBMC was plated in 96-well microplate precoated with swine specific IFN- γ antibody (10 μ g/mL, MABTECH). Cells were stimulated with challenging PRRSV strains at multiplicity of infection

(MOI) of 0.01 as the recall antigen for 20 h incubation at 37 °C in a 5% CO₂ atmosphere. Unstimulated cells and phytohemagglutinin (10 μ g/mL)-stimulated cells were used as negative and positive controls, respectively (Meier et al., 2003; Diaz et al., 2005; Diaz and Mateu, 2005; Park et al., 2014). The spots on the membranes were read by an automated ELISPOT Reader (AID ELISPOT Reader, AID GmbH, Strassberg, Germany). The results were expressed as the numbers of IFN- γ -SC per 10⁶ PBMC.

2.7. Pathology and immunohistochemistry

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 (Halbur et al., 1995). Microscopic lung lesion and immunohistochemistry (IHC) were performed on three blocks of lung tissues, which included eight pieces of lung: two piece from the right cranial lobe, two from the right middle lobe, one from the ventromedial part of the right caudal lobe, one from the dorsomedial part of the right caudal lobe, one from the midlateral part of the right caudal lobe, and one from the accessory lobe of each pig. The choice of lung tissues was based on the presence of macroscopic lesions. Microscopic lung lesions were scored blindly on a scale from 0 (normal) to 4 (severe diffuse) by two pathologists (Halbur et al., 1995).

IHC was performed using SR30 monoclonal antibody (Rural Technologies Inc., Brookings, SD, USA) as previously described (Han et al., 2012). SR30 monoclonal antibody (Rural Technologies

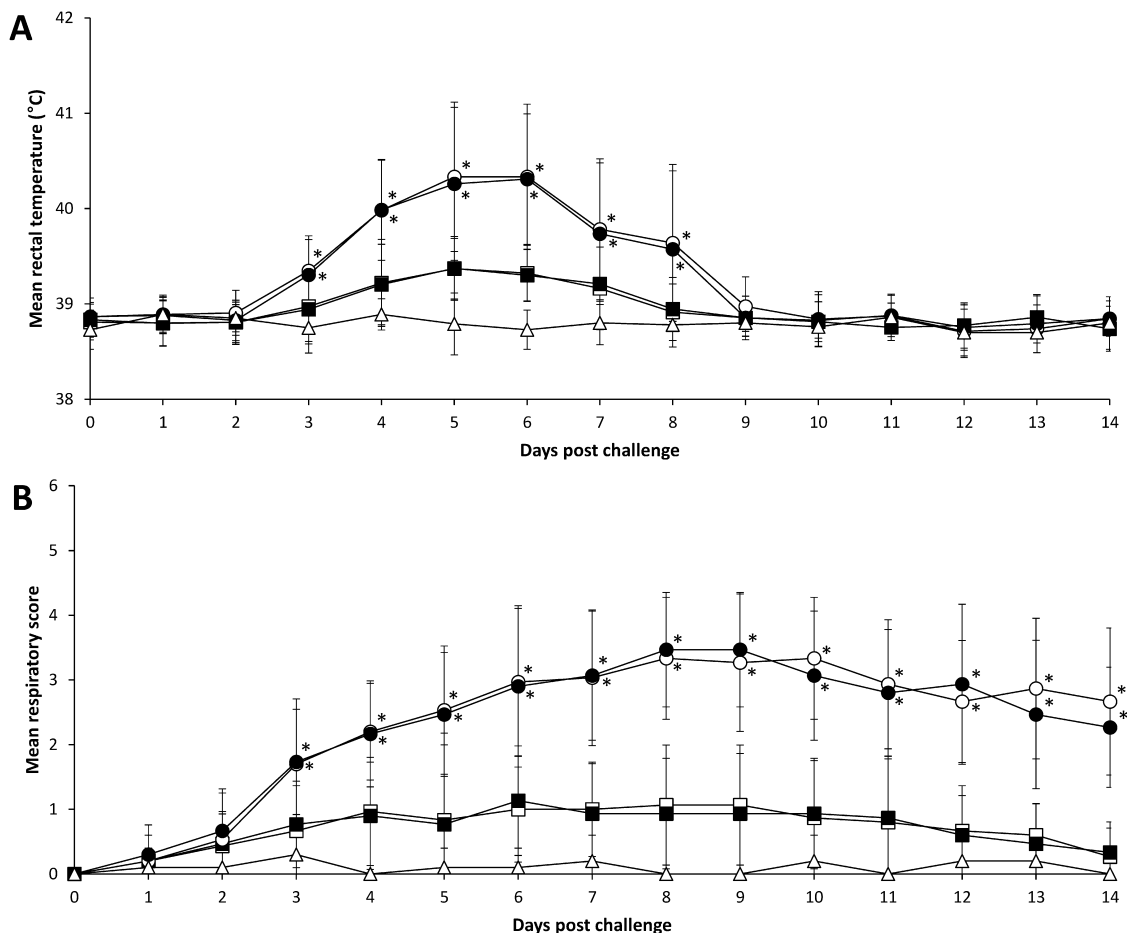


Fig. 1. Mean rectal temperature (A) and mean respiratory score (B) in Vac/Ch2L1 (□), Vac/Ch2L5 (■), UnVac/Ch2L1 (○), UnVac/Ch2L5 (●), and UnVac/UnCh (△). Variation is expressed as the standard deviation. The asterisks indicate significant differences ($P < 0.05$) between treatment and negative control group.

Inc.), capable of specifically recognizing nucleocapsid protein of PRRSV, was diluted 1:1000 in PBS (0.01 M, pH 7.4) containing 0.1% Tween 20. Three sections were cut from each of three blocks of tissue from one entire pulmonary lobe of each pig. In each slide, 10 fields were randomly selected, and the number of positive cells per unit area (0.95 mm²) was analyzed with the NIH Image J 1.45 s Program (<http://imagej.nih.gov/ij/download.html>) (Halbur et al., 1996a). The mean values were also calculated.

2.8. Phylogenetic analysis of the ORF5 gene

Because PRRSV was first isolated in 1995 in Korea, the present study analyzed the genetic variation of all GenBank-registered PRRSV using the nucleotide and amino acid sequences of PRRSV strains isolated in the first 5 years (1995–1999) and the most recent 5 years (2011–2015) in Korea. PRRSV PL97-1 strain (GenBank no. AY585241), isolated in 1997, is the first GenBank-registered PRRSV strain in Korea. Full ORF5 gene sequences from 19 Korean PRRSV isolates along with reference VR-2332 (GenBank no. U87392) and Ingelvac PRRSV MLV (GenBank no. AF066183) obtained from GenBank were aligned using ClustalW (Thompson et al., 1994). Subsequently, phylogenetic trees were automatically generated using Mega 6 software (Tamura et al., 2011). Bootstrap values were calculated on 1000 replicates of the alignment (Tamura et al., 2011).

2.9. Statistical analysis

Prior to statistical analysis, real-time PCR and NAs data were transformed to log₁₀ and log₂ values, respectively. Continuous data (rectal temperature, PRRSV RNA, serology, macroscopic lung lesions, and IFN- γ -SC) were analyzed using a repeated measures analysis of variance (ANOVA) for each time point. When the repeated measures ANOVA revealed significance, a one-way ANOVA was performed to determine the significance of individual between group differences. Discrete data (respiratory sign, macroscopic and microscopic lung lesions, and PRRSV-antigen) were analyzed by the non-parametric Kruskal–Wallis and Mann–Whitney test at each time point. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Clinical observation

Pigs in Vac/Ch2L1, Vac/Ch2L5, and UnVac/UnCh remained normal throughout the study, as measured by their respiratory scores and rectal temperatures. After the pigs were challenged with type 2 PRRSV, pigs in UnVac/Ch2L1 and UnVac/Ch2L5 developed fevers (ranging from 40 to 40.5 °C), and increased respiratory scores. Pigs in UnVac/Ch2L1 and UnVac/Ch2L5 had significantly higher ($P < 0.05$) rectal temperatures compared to pigs in Vac/Ch2L1, Vac/Ch2L5, and UnVac/UnCh from 3 to 8 dpc (Fig. 1A). Pigs in UnVac/Ch2L1 and UnVac/Ch2L5 had significantly higher ($P < 0.05$) respiratory clinical scores compared to pigs in Vac/Ch2L1, Vac/Ch2L5, and UnVac/UnCh from 3 to 14 dpc (Fig. 1B).

3.2. Quantification of PRRSV RNA in sera

No genomic copies of PRRSV was detected in the serum of any pig at the time of vaccination (–35 dpc). Log₁₀ transformed genomic copies of the vaccine strain were detected in the sera of vaccinated/challenged pigs (Vac/Ch2L1 and Vac/Ch2L5) from –28 to 0 dpc. Thereafter, no log₁₀ transformed genomic copies of vaccine strain were detected in the sera of vaccinated/challenged pigs. Log₁₀ transformed genomic copies of challenge type 2 PRRSV RNA were detected in the sera of pigs from Vac/Ch2L1, Vac/Ch2L5, UnVac/Ch2L1, and UnVac/Ch2L5 between 3 and 14 dpc. Pigs from Vac/Ch2L1 and Vac/Ch2L5 had significantly lower ($P < 0.05$) log₁₀ transformed genomic copies of challenge PRRSV RNA in their sera at 3, 7, 10, and 14 dpc compared to pigs from UnVac/Ch2L1 and UnVac/Ch2L5. However, there were no significant differences between Vac/Ch2L1 and Vac/Ch2L5 in terms of their log₁₀ transformed genomic copies of challenge PRRSV RNA throughout the experiment. No PRRSV was detected in the sera of pigs from UnVac/UnCh throughout the experiment (Fig. 2).

3.3. Serology

At the time of PRRSV vaccination (3 weeks of age; –35 dpc), pigs in all 5 groups were seronegative. Antibodies specific for the PRRSV were detected by ELISA in vaccinated challenged pigs (Vac/Ch2L1

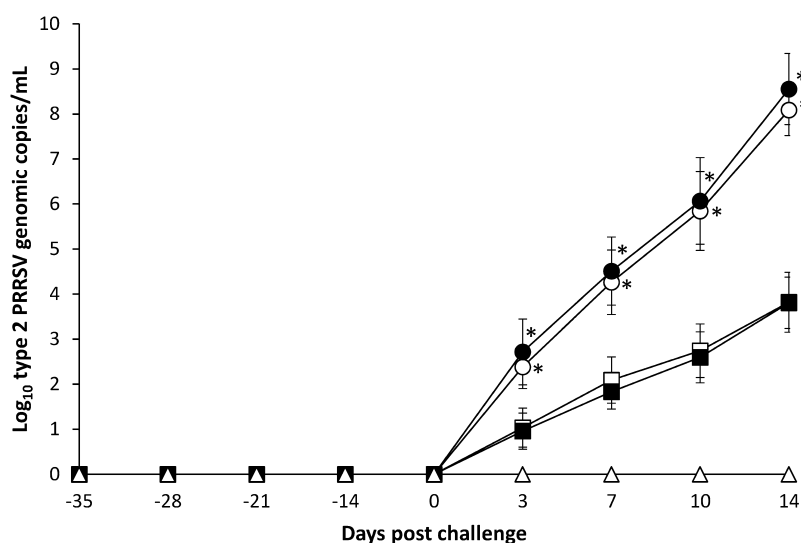


Fig. 2. Mean values of the genomic copy number of challenge type 2 PRRSV RNA in serum in Vac/Ch2L1 (□), Vac/Ch2L5 (■), UnVac/Ch2L1 (○), UnVac/Ch2L5 (●), and UnVac/UnCh (△). Variation is expressed as the standard deviation. The asterisks indicate significant differences ($P < 0.05$) between treatment and negative control group.

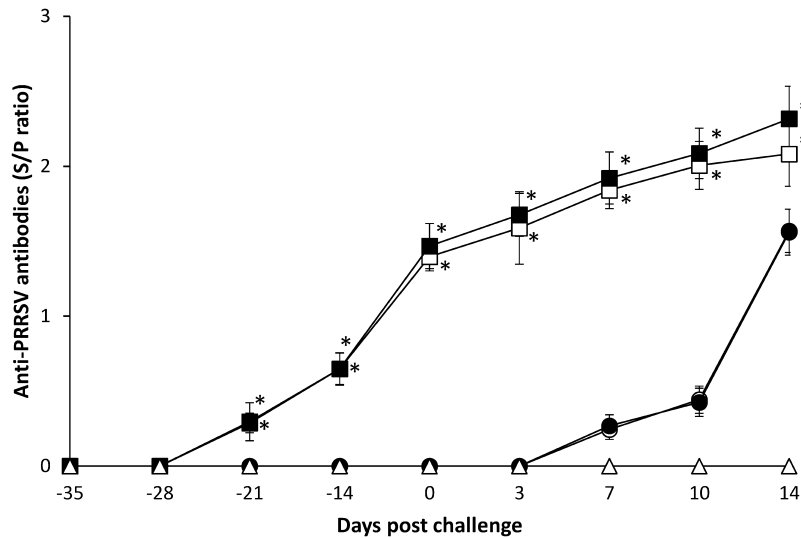


Fig. 3. Mean values of the anti-PRRSV antibodies in Vac/Ch2L1 (□), Vac/Ch2L5 (■), UnVac/Ch2L1 (○), UnVac/Ch2L5 (●), and UnVac/UnCh (△). Variation is expressed as the standard deviation. The asterisks indicate significant differences ($P < 0.05$) between treatment and negative control group.

and Vac/Ch2L5) from -21 dpc (14 days post-vaccination) onward and in unvaccinated challenged pigs (UnVac/Ch2L1 and UnVac/Ch2L5) from 7 dpc onward. Anti-PRRSV antibody titers were significantly higher ($P < 0.05$) in vaccinated challenged pigs (Vac/Ch2L1 and Vac/Ch2L5) compared to unvaccinated challenged (UnVac/Ch2L1 and UnVac/Ch2L5) pigs between -21 and 14 dpc. Anti-PRRSV antibody titers were not detected in pigs from UnVac/UnCh at any time (Fig. 3).

Homologous (against the vaccine strain) and heterologous (against the challenge virus) NAs titers were not detected in any pigs from any group (NAs titers $< 2 \log_2$) throughout the experiment.

3.4. Interferon- γ secreting cells

Upon challenge with PRRSV (SNUVR150004 strain), pigs from Vac/Ch2L1 had significantly higher ($P < 0.05$) numbers of virus-specific IFN- γ -SC in PBMC compared to pigs from UnVac/Ch2L1 at 3, 7, 10, and 14 dpc. Upon challenge with PRRSV (SNUVR150324

strain), pigs from Vac/Ch2L5 had significantly higher ($P < 0.05$) numbers of virus-specific IFN- γ -SC in PBMC compared to pigs from UnVac/Ch2L5 at 3, 7, 10, and 14 dpc. No PRRSV-specific IFN- γ -SC was detected in pigs from UnVac/UnCh throughout the experiment (Fig. 4).

3.5. Pathology

Macroscopic lung lesions were observed primarily in the cranial, middle, and ventromedial portion of the caudal lung lobes. The affected lungs often failed to collapse and the parenchyma was more firm and heavy compared to negative control pig. No macroscopic lung lesions were observed in the negative control pigs in UnVac/UnCh. Pigs in UnVac/Ch2L1 and UnVac/Ch2L5 had significantly higher ($P < 0.05$) macroscopic lung lesion scores compared to pigs in Vac/Ch2L1, Vac/Ch2L5, and UnVac/UnCh at 7 and 14 dpc (Table 1).

The microscopic lung lesions were characterized by septal thickening with mononuclear cells and accumulation of

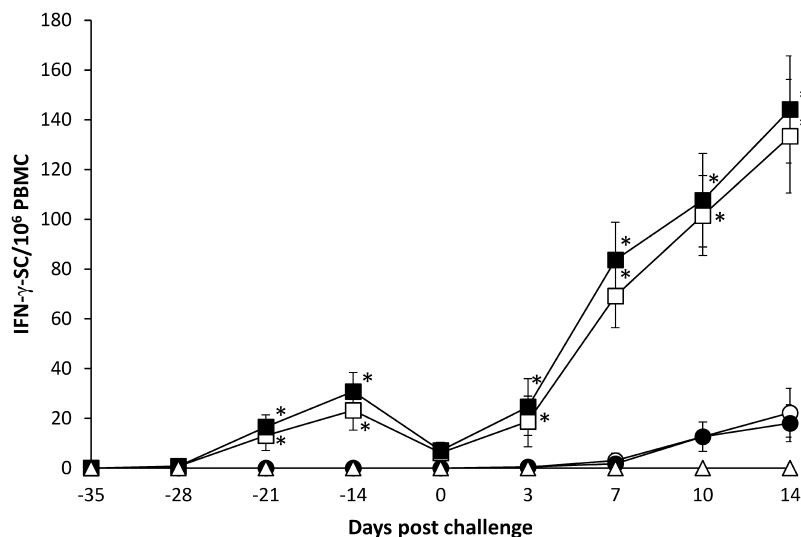


Fig. 4. Frequency of challenge type 2 PRRSV-specific IFN- γ -SC/10⁶ PBMC in Vac/Ch2L1 (□), Vac/Ch2L5 (■), UnVac/Ch2L1 (○), UnVac/Ch2L5 (●), and UnVac/UnCh (△). Variation is expressed as the standard deviation. The asterisks indicate significant differences ($P < 0.05$) between treatment and negative control group.

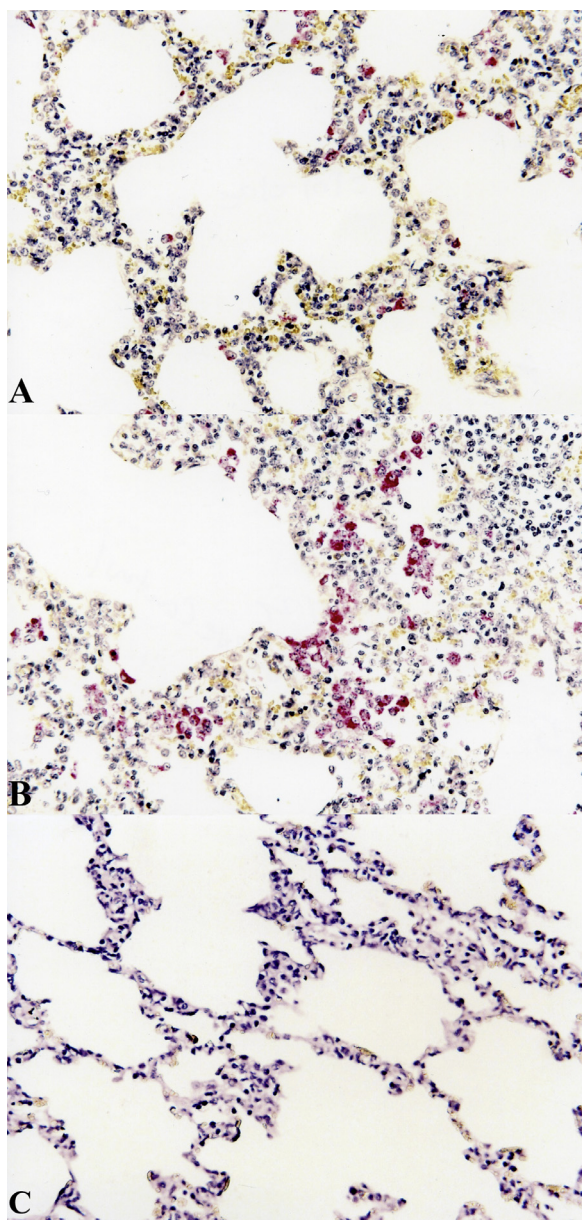


Fig. 5. Immunohistochemistry for the detection of type 2 PRRSV antigen in the lungs from Vac/Ch2L1 (A), UnVac/Ch2L1 (B), and UnVac/UnCh (C). Pigs in UnVac/Ch2L1 (B) had significantly higher ($P < 0.05$) PRRSV-positive cells scores compared to pigs in Vac/Ch2L1 (A) and UnVac/UnCh (C) at 7 dpc.

macrophages in alveolar spaces. The lesions were often multifocal in distribution. No microscopic lung lesions were observed in the negative control pigs in UnVac/UnCh. Pigs in UnVac/Ch2L1 and UnVac/Ch2L5 had significantly higher ($P < 0.05$) microscopic lung lesion scores compared to pigs in Vac/Ch2L1, Vac/Ch2L5, and UnVac/UnCh at 7 and 14 dpc (Table 1).

PRRSV antigen was detected by immunohistochemistry in the lungs of pigs from Vac/Ch2L1, Vac/Ch2L5, UnVac/Ch2L1, and UnVac/Ch2L5 throughout the experiment (Table 1). PRRSV antigens were detected exclusively within the cytoplasm of macrophages and pneumocytes. Pigs in Vac/Ch2L1 (Fig. 5A), Vac/Ch2L5, and UnVac/UnCh had significantly lower ($P < 0.05$) PRRSV-positive cells scores compared to pigs in UnVac/Ch2L1 (Fig. 5B) and UnVac/Ch2L5 at 7 and 14 dpc. No immunohistochemical staining for PRRSV antigen was observed in lung sections from negative control pigs in UnVac/UnCh (Fig. 5C).

3.6. Phylogenetic analysis of the ORF5 gene

The 19 PRRSV isolates showed a nucleotide homology ranging from 82.2 to 100%, and an amino acid homology ranging from 83 to 100% (Fig. 6). The full ORF5 gene sequence from SNUVR150004 (GenBank no. KU301047) exhibited 84.9% (85%), 84.9% (84.5%), and 84.9% (84.5%) of nucleotide (amino acid) homology with PL97-1 strain (1997 isolate, GenBank no. AY585241), VR-2332 strain, and Ingelvac PRRS MLV vaccine virus, respectively. The full ORF5 gene sequence from SNUVR150324 (GenBank no. KU301048) exhibited 99.1% (98.5%), 99.5% (99%), and 99.1% (98.5%) of nucleotide (amino acid) homology with PL97-1 strain (1997 isolate, GenBank no. AY585241), VR-2332 strain, and Ingelvac PRRS MLV vaccine virus, respectively.

4. Discussion

After 20 years of use in over 35 million pigs (<http://www.bivkorea.com>), PRRS MLV used in this study is still proven to be efficacious against recently isolated heterologous type 2 PRRSV strains. These results provide swine practitioners and producers with clinically significant information because there have been concerns about the efficacy of this PRRS MLV over the massive use for 20 years. Genetic variation of 1995–1999 and 2011–2015 Korean isolates was determined based on ORF5, which encodes for highly variable regions (Murtaugh et al., 1995). ORF5 of Korean PRRSV isolates showed 82.2–100% homology at nucleotide level and 83–100% at amino acid level. 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). Despite the fact that genetic variation may affect antigenic diversity (Frossard et al., 2012), present results suggest that PRRS MLV is still closely related antigenically to the recently isolated field strains.

The most common and representative strains of PRRSV were chosen to evaluate the PRRS MLV used in the present study. Two challenge viruses (SNUVR150004 and SNUVR150324) are local field isolates. The severity of experimentally reproduced clinical respiratory disease and lesions was generally consistent with the clinical respiratory disease observed on the farm from which each strain originated. Two challenge viruses are high virulent as other Korean type 2 PRRSV isolate (SNUVR090851, GenBank JN315685) based on levels of viremia and lung lesion (Han et al., 2013). Genetically, global type 2 diversity is dominated mainly by isolates of four lineages 1, 5, 8, and 9, which collectively constitute over 97% of all ORF5 sequences (Brar et al., 2015). Among those, type 2 PRRSV belonging to lineages 1 and 5 are the most commonly identified in Korea (Shi et al., 2010). Korean type 2 field strains belonging to lineage 5 shared a high degree of identity to the PRRS MLV vaccine virus which is based on the VR2332 isolate (Cheon and Chae, 2000). At the present time, the origin of Korean PRRS MLV-like type 2 PRRSV field strains has not been determined. It is possible that these field strains prevailed in Korea before the introduction of the PRRS MLV or may have originated from the vaccine virus.

Although the precise protective immunity induced by PRRS vaccine remains unknown, NAs and IFN- γ are the most studied immune mechanisms of protection against PRRSV (Kimman et al., 2009; Murtaugh and Genzow, 2011). Passive transfer of a high titer of PRRSV-specific NAs (1:32) protected pigs against respiratory disease and even then, apparent sterilizing immunity was attained in 50% of the animals (Lopez et al., 2007). However, NAs are not able to play an important role in viral clearance because PRRSV viremia is often resolved even before NAs are detected in infected (Nelson et al., 1994; Mateu and Diaz, 2008) and vaccinated pigs (Mengeling et al., 2003) confirmed by the present study. In contrast

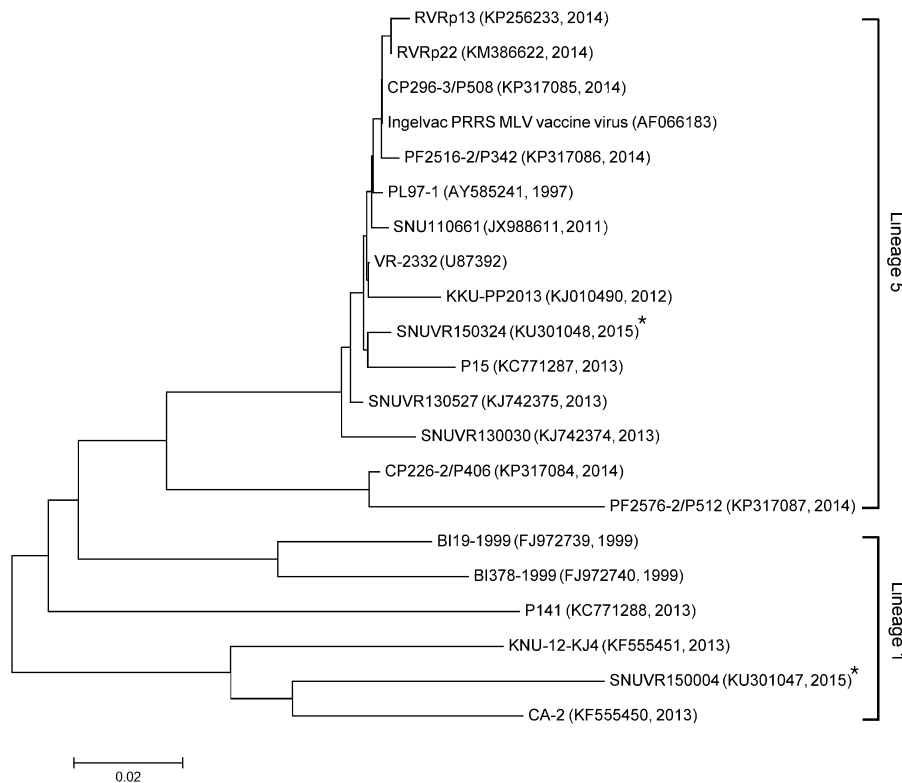


Fig. 6. Phylogenetic tree of the nucleotide sequences for full ORF5 genes of the 19 Korean PRRSV strains isolated in the first 5 years (1995–1999) and the most recent 5 years (2011–2015) in Korea, and related reference virus. Phylogenetic tree was constructed by the neighbor-joining method. GenBank number was listed in the phylogenetic tree. The asterisks indicate two Korean challenge viruses (SNUVR150004 and SNUVR150324) used in this study.

to NAs, the apparently simultaneous reduction of PRRSV viremia and induction of PRRSV-specific IFN- γ -SC was observed in vaccinated/challenged pigs during early viremic phase of PRRSV infection. Our data are interpreted as an indicator of the ability of IFN- γ -SC to play a role in protection against PRRSV infection. Nevertheless, in another study, the significance of IFN- γ -SC is not known since there is no association with control of infection (Xiao et al., 2004). These results suggest that PRRSV-specific IFN- γ -SC is not solely responsible for the protection against PRRSV infection.

Pathological evaluation is critical in determining the efficacy of a PRRS vaccine because PRRSV causes interstitial pneumonia in growing pigs. Type 2 PRRSV strains used in this study induce respiratory symptoms and lung lesions in unvaccinated challenged pigs. In contrast, the vaccination of pigs against PRRSV significantly reduced interstitial pneumonia and the PRRSV antigen within lung lesions in vaccinated/challenged pigs, regardless of PRRSV genotypes. These pathological data confirm the good efficacy of the PRRS MLV.

To the authors' knowledge, this is the first study about the evaluation of this 20 year old PRRS MLV against recently isolated PRRSV field strains. Much effort has been made to control PRRS disease in Korea in the last two decades. Regular evaluation of PRRS MLV provides clinically significant information for practitioners and producers because vaccination of pigs with PRRSV has been commonly used to control PRRSV infection in Korea.

Competing interests

None.

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