



Cross-protection of a new type 2 porcine reproductive and respiratory syndrome virus (PRRSV) modified live vaccine (Fostera PRRS) against heterologous type 1 PRRSV challenge in growing pigs



Changhoon Park¹, Kyuhyung Choi¹, Jiwoon Jeong, Chanhee Chae^{*}

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

ARTICLE INFO

Article history:

Received 3 January 2015

Received in revised form 15 February 2015

Accepted 17 February 2015

Keywords:

Cross-protection

Genotype

Porcine reproductive and respiratory syndrome virus

Vaccine

ABSTRACT

The objective of the present study was to determine the cross-protection of a new type 2 porcine reproductive and respiratory syndrome virus (PRRSV) modified live vaccine against heterologous type 1 PRRSV challenge in growing pigs. The mean rectal temperature and respiratory score was significantly ($P < 0.05$) lower in vaccinated challenged pigs than in unvaccinated challenged pigs. Vaccination of pigs with type 2 PRRSV reduced the levels of type 1 PRRSV viremia after challenge with type 1 PRRSV. Vaccinated challenged pigs had significantly ($P < 0.05$) higher frequency of interferon- γ secreting cells and lower levels of interleukin-10 compared to unvaccinated challenged pigs. Vaccination of pigs with the type 2 PRRSV effectively reduced the macroscopic and microscopic lung lesion and the type 1 PRRSV antigens within lung lesions in vaccinated challenged pigs. This study demonstrates partial cross-protection of a new type 2 PRRSV modified live vaccine against heterologous type 1 PRRSV challenge in growing pigs.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped positive-strand RNA virus classified in the order *Nidovirales*, family *Arteriviridae*, and genus *Arterivirus* (Snijder and Meulenbergh, 1998). PRRSV was first isolated in the Netherlands in 1991 (Wensvoort et al., 1991) and subsequently in the United States in 1992 (Collins et al., 1992). In the 1990s, all European PRRSV isolates were closely related and designated as the type 1 (European) genotype. Type 1 PRRSVs

are further divided into three subtypes based on the open reading frame (ORF) 7 size: a pan-European subtype 1 and East European subtypes 2 and 3 with nucleocapsid protein sizes of 128, 125 and 124 amino acids, respectively (Stadejek et al., 2008). All North American isolates were also closely related and designated as the type 2 (North American) genotype (Allende et al., 1999; Murtaugh et al., 2010). Type 1 and type 2 PRRSVs differ significantly in terms of their clinical, genetic, and antigenic properties (Kapur et al., 1996; Labarque et al., 2004; Nelson et al., 1993).

PRRSV is one of the most important challenges currently facing the global swine industry. A PRRSV infection is clinically characterized by reproductive failure in sows and respiratory disease in growing pigs of all ages, causing huge economic losses (Zimmerman et al., 2012). In

^{*} Corresponding author. Tel.: +82 2880 1277; fax: +82 2871 5821.

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

¹ These authors contributed equally to this work.

Korea, the first case of PRRSV infection was reported in 1994 (Kweon et al., 1994). All PRRSV isolates corresponded to type 2 PRRSV until 2000 (Cheon and Chae, 2000). Currently, the type 1 PRRSV has also emerged, and both genotypes circulate concurrently in Asian countries, including Korea (Chen et al., 2011; Nam et al., 2009; Thanawongnuwech et al., 2004).

Cross-protection conferred by type 2 PRRSV vaccine against type 1 PRRSV is an important clinical issue in many Asian countries because of the emergence of type 1 PRRSV. In previous cross-protection studies, type 2 PRRSV modified live vaccine have not been effective when applied to control pigs against type 1 PRRSV isolated from European countries (Labarque et al., 2003; van Woensel et al., 1998; Labarque et al., 2000). Recently, a new type 2 PRRSV modified live vaccine (Fostera PRRS, Zoetis, Florham Park, NJ, USA) was registered in Korea in 2014. This new type 2 PRRSV vaccine is efficacious to protect pigs against heterologous type 2 PRRSV challenge (Park et al., 2014). However, whether there is cross-protection from the same vaccine against heterologous type 1 PRRSV challenge is not currently known. Hence, the objective of this study was to evaluate the efficacy of a new type 2 PRRSV vaccine against heterologous type 1 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 inoculum

Type 1 PRRSV (SNUVR090485, pan-European subtype 1) was used as inoculum. SNUVR090485 virus (GenBank no. JN315686 and JN399073) was isolated from lung samples of an aborted fetus and a weaned pig in a 1000-sow herd in southwestern Kyunggi Province (Han et al., 2012a). The SNUVR090485 is subtype 1 based on ORF7 analysis (Stadejek et al., 2008) and vaccine strain P129 is lineage 8 based on ORF5 analysis (Shi et al., 2010). The SNUVR090485 and vaccine strains (P129) share 53.8% and 60.3% amino acid identity for ORF5 and ORF7, respectively.

2.2. Experimental design

A total of 148 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 viremia by real-time polymerase chain reaction (PCR).

Pigs were divided into three groups using the random number generation function (Excel, Microsoft Corporation, Redmond, WA, USA). The pigs in group 1 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. At 56 days of age (0 days post challenge, dpc), the pigs ($n=60$ /group) in groups 1 and 2 were inoculated intranasally with 3 mL of tissue culture fluid containing 10^5 50% tissue culture infective dose (TCID₅₀)/mL of PRRSV (SNUVR090485 strain, fourth passage in alveolar

macrophages) 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 ($n=28$) in group 3 remained unvaccinated unchallenged and served as the negative control group. The pigs in each group were housed separately within the facility. Blood samples and nasal swabs were collected at –35, –32, –30, –28, –25, –21, –14, 0, 7, 10, 14, and 21 dpc. Subsets of pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 7, 10, 14, and 21 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 Seoul National University Institutional Animal Care and Use, and Ethics Committee.

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 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 the type 1 and type 2 PRRSV were performed to quantify PRRSV genomic cDNA copy (Wasilk et al., 2004). Real-time PCR for the vaccine strain was also performed to quantify PRRSV genomic cDNA copy (Park et al., 2014). A sample was considered positive if the cycle threshold level was obtained at ≤ 40 cycles (Chung et al., 2005).

2.5. Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturer's instructions. Serum samples were considered positive for PRRSV antibody if the S/P ratio was greater than 0.4. Serum virus neutralization (SVN) tests were also performed with the challenging strain, type 1 PRRSV (SNUVR090485), as previously described (Yoon et al., 1994). The presence of virus-specific cytopathic effect (CPE) in each well was recorded after incubating for 7 days. 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 PRRSV-specific interferon- γ secreting cells (IFN- γ -SC) using challenging type 1 PRRSV were determined in peripheral blood mononuclear cells (PBMC) as previously described (Diaz and Mateu, 2005; Meier et al., 2003; Park et al., 2014).

2.7. Quantification of interleukin-10 secretion

The protein levels of interleukin-10 (IL-10) were quantified in the supernatants of PBMC (2×10^6 cells per well, 250 μ L) cultures in vitro for 20 h with the challenging type 1 PRRSV (MOI 0.01) or PHA (10 μ g/mL) by using commercial ELISA kits (Swine Interleukin-10 ELISA kit, Invitrogen, Camarillo, CA, USA) according to the manufacturer's instructions. Detection limits for IL-10 were 3.0 pg/mL.

2.8. Pathology and immunohistochemistry

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

2.9. Virus isolation

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

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, IL-10, PRRSV antigen score, and macroscopic lung lesion score) were analyzed with Student *t* test at each time point. Discrete data (microscopic lung lesion score and respiratory score) were analyzed by Mann–Whitney tests. Chi-square and Fisher's exact test were applied to evaluate the proportion of viremic and nasal shedding pigs. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Clinical observation

The vaccinated challenged pigs (group 1) remained normal throughout the study, as measured by their respiratory scores and rectal temperatures. The mean rectal temperature was significantly ($P < 0.05$) higher in unvaccinated challenged pigs than in pigs in the other 2 groups at 3 and 4 dpc (Fig. 1A). The mean respiratory scores were significantly ($P < 0.05$) higher in unvaccinated challenged pigs than in pigs in the other two groups at 6, 7, 8, and 9 dpc (Fig. 1B). Negative control pigs maintained normal temperatures without respiratory signs 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 sera from the vaccinated challenged pigs at –32 (3 days

post vaccination), –30, –28, and –25 dpi but, thereafter, no vaccine strain was detected in the sera from pigs in this group throughout the experiment. Genomic copies of the type 1 PRRSV were detected in the serum samples of vaccinated challenged (group 1) and unvaccinated challenged (group 2) pigs after inoculation with this virus.

Vaccinated challenged pigs had a significantly ($P < 0.05$) lower number of genomic copies of type 1 PRRSV in their blood compared to unvaccinated challenged pigs at 7, 10, 14, and 21 dpc (Fig. 2). Challenging type 1 PRRSV was detected in the blood of vaccinated challenged pigs at 7 and 10 dpc. The prevalence of viremic pigs was significantly lower in the vaccinated challenged pigs (10/60 at 7 dpc, 1/45 at 10 dpc, 0/30 at 14 dpc, and 0/15 at 21 dpc) compared with the unvaccinated challenged pigs (60/60 at 7 dpc, 42/45 at 10 dpc, 24/30 at 14 dpc, and 8/15 at 21 dpc).

3.3. Anti-PRRSV antibodies

At the time of PRRSV vaccination (3 weeks of age; –35 dpc), pigs in all three groups were seronegative. Antibodies specific for PRRSV were detected by ELISA in vaccinated challenged pigs from –25 dpc (10 days post vaccination) onward and in unvaccinated challenged pigs from 10 dpc onward. Vaccinated challenged pigs induced significantly ($P < 0.05$) higher anti-PRRSV antibody titers than that in unvaccinated challenged pigs from –25 to 21 dpc (Fig. 3). 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

After stimulation with the type 1 PRRSV, the frequency of type 1 PRRSV-specific IFN- γ -SC/ 10^6 PBMC reached an average of 21.2 ± 12.3 cells/ 10^6 PBMC at –21 dpc in vaccinated challenged pigs. Upon challenge with type 1 PRRSV, the frequency of type 1 PRRSV-specific IFN- γ -SC increased gradually and reached an average of 70.6 ± 13.6 cells/ 10^6 PBMC at 21 dpc in vaccinated challenged pigs. Vaccinated challenged pigs produced significantly ($P < 0.05$) higher numbers of IFN- γ -SC at 7, 10, 14, and 21 dpc compared to unvaccinated challenged pigs (Fig. 4A). The mean frequencies of type 1 PRRSV-specific IFN- γ -SC remained at basal levels (< 20 cells/ 10^6 PBMC) in the negative control pigs at any time.

3.6. Interleukin-10

Upon challenge with type 1 PRRSV, the IL-10 reached maximal levels at 7 dpc and, thereafter, decreased gradually until 14 dpc in vaccinated challenged and unvaccinated challenged pigs. Unvaccinated challenged pigs had significantly higher levels of IL-10 at 7 and 10 dpc compared to vaccinated challenged pigs (Fig. 4B). IL-10 was not detected in negative control pigs throughout the experiment.

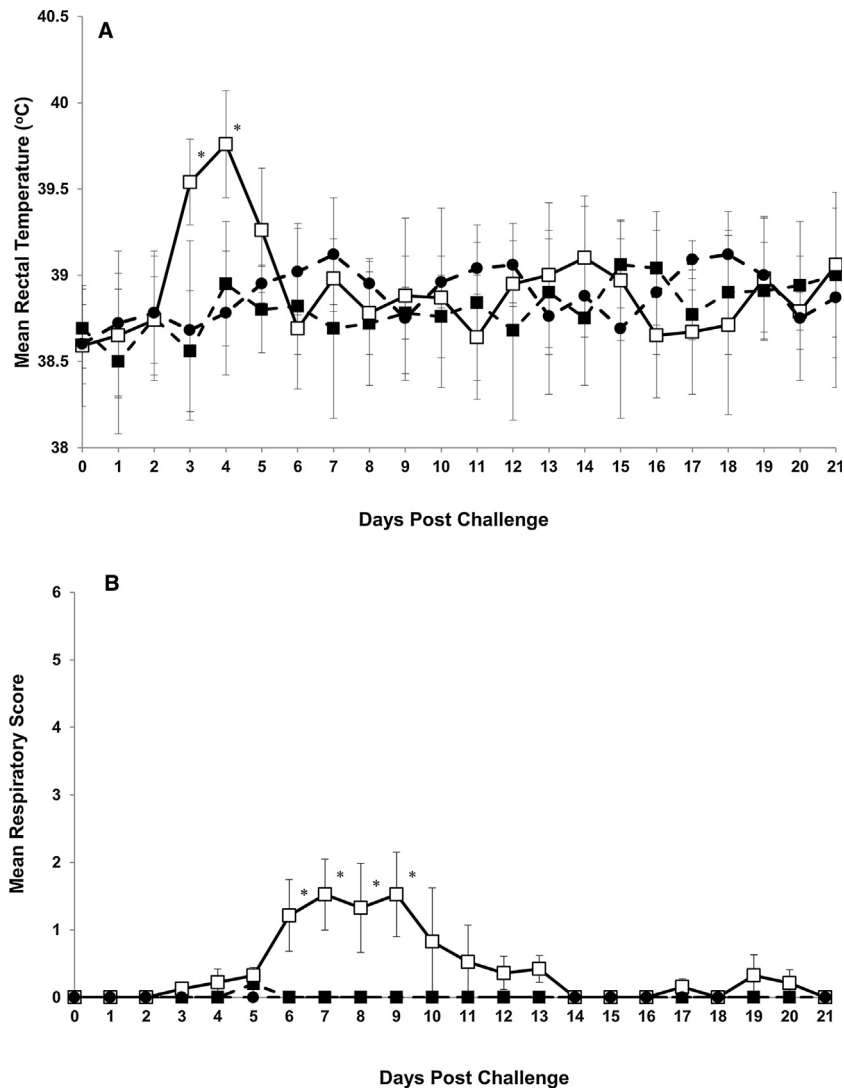


Fig. 1. Mean rectal temperatures (A) and mean respiratory score (B) in vaccinated challenged (■), unvaccinated challenged (□), and negative control pigs (●). Significant difference is indicated at P value $< 0.05^*$.

3.7. Macroscopic and microscopic lung lesions

Lung lesions were observed in vaccinated challenged and unvaccinated challenged pigs but negative control pigs were normal. The lesions were present predominantly in the middle, caudal and accessory lobes. Macroscopic lung lesions were characterized by multifocal, tan-mottled areas with irregular and indistinct borders. Microscopic lung lesions were characterized by thickened alveolar septa with increased numbers of interstitial macrophages and lymphocytes and by type II pneumocyte hyperplasia. The mean macroscopic lung lesion scores were significantly ($P < 0.05$) lower in the vaccinated challenged pigs than in the unvaccinated challenged pigs at 7, 10, and 14 dpc (Table 1). The mean microscopic lung lesion scores were significantly ($P < 0.05$) lower in the vaccinated challenged pigs than in the unvaccinated challenged pigs at 7, 10, 14, and 21 dpc (Table 1).

3.8. 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 ($P < 0.05$) lower in vaccinated challenged pigs than in unvaccinated challenged pigs at 7, 10, 14, and 21 dpc (Table 1, Fig. 5). No staining for PRRSV antigen was observed in lung sections from the negative control pigs.

3.9. Virus isolation

PRRSV was isolated from the lungs at different dpc. The type 1 PRRSV isolated from vaccinated challenged pigs (15/15 at 7 dpc, 10/15 at 10 dpc, 7/15 at 14 dpc, and 5/15 at 21 dpc) and unvaccinated challenged pigs (15/15 at 7, 10,

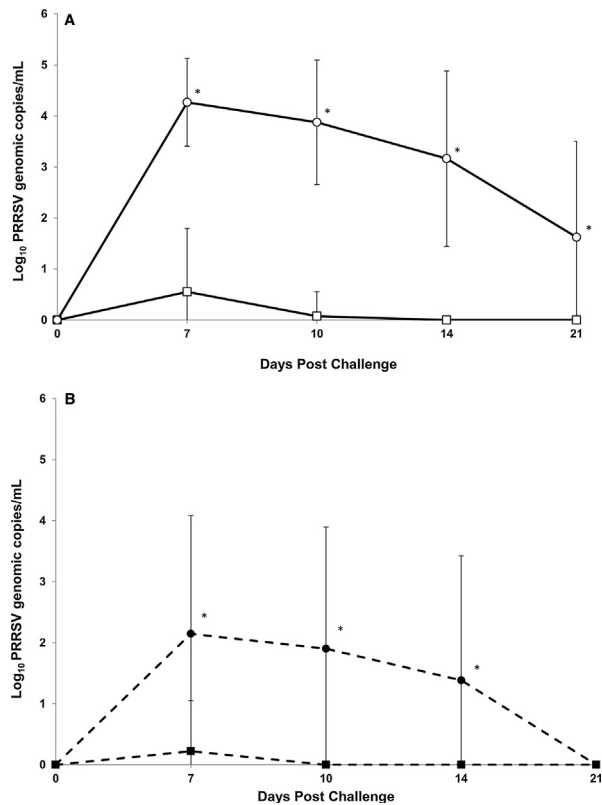


Fig. 2. Mean values of the genomic copy number of PRRSV RNA in serum in vaccinated challenged (■) for serum and (■) for nasal swab) and unvaccinated challenged (□) for serum and (●) for nasal swab) pigs. Variation is expressed as the standard deviation. Significant difference is indicated at P value $<0.05^*$.

14, and 21 dpc) was confirmed to be the same as the challenge virus through sequence analysis. No type 2 PRRSV or vaccine virus was isolated from vaccinated challenged and unvaccinated challenged pigs. No PRRSV was isolated from negative control pigs.

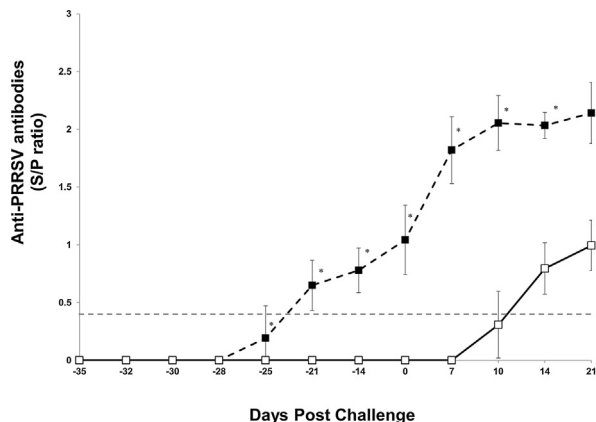


Fig. 3. Mean values of the anti-PRRSV antibodies in the different groups. Vaccinated challenged (■) and unvaccinated challenged (□) pigs. Variation is expressed as the standard deviation. Significant difference is indicated at P value $<0.05^*$.

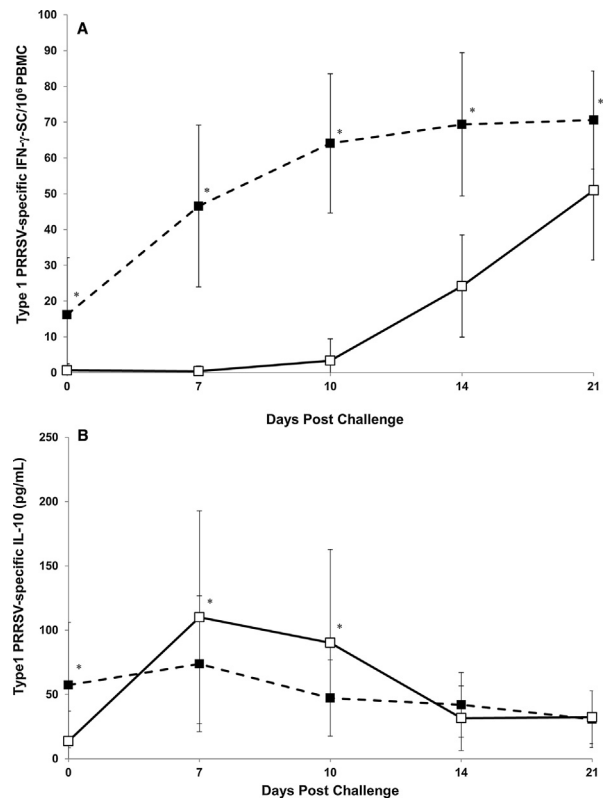


Fig. 4. Frequency of challenging type 1 PRRSV-specific IFN- γ -SC/10⁶ PBMC (A) and level of interleukin-10 (B). Vaccinated challenged (■) and unvaccinated challenged (□) pigs. Variation is expressed as the standard deviation. Significant difference is indicated at P value $<0.05^*$.

4. Discussion

The results of the present study demonstrate that a new type 2 PRRSV vaccine is efficacious in protecting growing pigs from respiratory disease caused by heterologous type 1 PRRSV challenge. Vaccination of pigs with type 2 PRRSV is able to reduce the levels of type 1 PRRSV viremia and nasal shedding, as well as the severity of lung lesions after heterologous type 1 PRRSV challenge. These results are in contrast with previous studies in which another type 2 PRRSV vaccine barely reduces the amount of viral load in blood and clinical signs after heterologous type 1 PRRSV challenge in pigs (Labarque et al., 2000, 2003; van Woensel et al., 1998). We have no clear explanation why different type 2 PRRSV vaccines have different results of cross-protection against type 1 PRRSV challenge. It may be due to differences between the vaccine strains or antigenic variation among challenging type 1 PRRSV strains. Different PRRSV isolates already show a remarkable antigenic variation (Kvisgaard et al., 2013).

Interestingly, there are differences in the ratio and duration of viremia in pigs vaccinated with same type 1 PRRSV vaccine followed by a challenge with either homotypic (type 2) or heterotypic (type 1) PRRSV. The ratio of viremic pigs in vaccinated groups challenged with homotypic virus in a previous study (Park et al., 2014) was

Table 1

Proportion of viremic pigs, macroscopic and microscopic lung lesion score, and immunohistochemical antigen score of porcine reproductive and respiratory syndrome virus (PRRSV) among three groups at different days post challenge (dpc).

	dpc	Groups		
		Vaccinated challenged	Unvaccinated challenged	Unvaccinated unchallenged
Proportion of viremic pigs	7	10/60 ^b	60/60 ^a	0/28 ^c
	10	1/45 ^b	42/45 ^a	0/21 ^b
	14	0/30 ^b	24/30 ^a	0/14 ^b
	21	0/15 ^b	8/15 ^a	0/7 ^b
Macroscopic lung lesion score	7	17.5 ± 5 ^b	32.5 ± 5 ^a	0 ^c
	10	17.5 ± 9.5 ^b	32.5 ± 5 ^a	0 ^c
	14	2.5 ± 5 ^b	15 ± 5.7 ^a	0 ^b
	21	0	10 ± 8.1	0
Microscopic lung lesion score	7	0.7 ± 0.24 ^b	1.77 ± 0.3 ^a	0 ^c
	10	0.27 ± 0.09 ^b	2.25 ± 0.54 ^a	0 ^c
	14	0.15 ± 0.12 ^b	1.37 ± 0.2 ^a	0 ^c
	21	0.02 ± 0.05 ^b	1.17 ± 0.67 ^a	0 ^b
PRRSV-antigen score	7	2.5 ± 2.9 ^b	13.9 ± 1.5 ^a	0 ^b
	10	0.3 ± 0.6 ^b	13.7 ± 5 ^a	0 ^b
	14	0.2 ± 0.5 ^b	9.1 ± 1.1 ^a	0 ^b
	21	0 ^b	4 ± 1.5 ^a	0 ^b

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

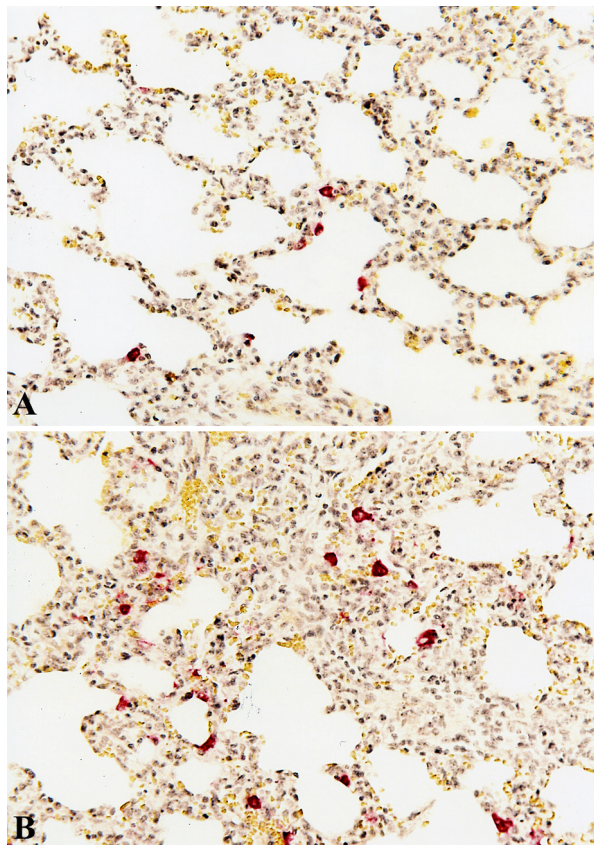


Fig. 5. Immunohistochemistry of PRRSV antigen. A few PRRSV antigens were detected in lungs with mild interstitial pneumonia in vaccinated challenged pig (A) and numerous PRRSV antigens were detected in lungs with severe interstitial pneumonia in unvaccinated challenged pigs (B) at 10 days post challenge.

higher than that in vaccinated groups challenged with heterotypic virus in the present study. In addition, viremic pigs were detected in vaccinated and homotypic challenged group until the 21 dpc in the previous study (Park et al., 2014). Viremic pigs were not found in vaccinated and heterotypic challenged group from 14 dpc onward in this study. Differences in the ratio and duration of viremia are due to the different virulence between the challenging type 1 and type 2 PRRSV. Respiratory infection by type 1 PRRSV is usually mild, and ratio and duration of viremia are relatively lower and shorter compared to type 2 PRRSV infection (Han et al., 2013b; Johnson et al., 2004; Labarque et al., 2003). Moreover, the ratio and duration of viremic pigs in unvaccinated groups challenged with type 2 PRRSV in the previous study (Park et al., 2014) was higher and shorter than that in unvaccinated groups challenged with type 1 PRRSV virus in the present study. Therefore, the difference in virulence between two genotypes explains the differences in ratio and duration of viremic pigs between vaccinated groups challenged with type 1 and type 2 PRRSV.

PRRSV viremia plays a central role in the development of respiratory disease (Han et al., 2013a). Therefore, reduction of PRRSV viremia is an important parameter to evaluate the efficacy of PRRSV vaccine (Labarque et al., 2003; van Woensel et al., 1998). Vaccination of pigs with type 2 PRRSV reduces levels of type 1 PRRSV viremia. In addition, the reduction of type 1 PRRSV viremia coincided with the appearance of type 1 PRRSV-specific IFN- γ -SC. PRRSV-specific IFN- γ -SC plays a critical role in viral clearance (Meier et al., 2003; Park et al., 2014). It is also known that IFN- γ inhibits PRRSV replication in alveolar macrophages (Bautista and Molitor, 1999; Rowland et al., 2001). These observations are in contrast with an experimental study in which the significance of IFN- γ -SC is not known since there is no association with control of infection (Xiao et al., 2004). Despite the fact that

induction of type 1 PRRSV-specific IFN- γ -SC by type 2 PRRSV vaccine may result in reduction of type 1 PRRSV viremia in the present study, further studies are needed to determine the protective role of IFN- γ -SC against PRRSV challenge.

IL-10 is a known potent immunosuppressive cytokine (Conti et al., 2003). In the present study, PRRSV-specific IFN- γ -SC gradually increased at the same time as the gradual decrease of IL-10, indicating that the activation of IFN- γ -SC may be modulated by the induction of IL-10, as previously noted (Diaz and Mateu, 2005; Suradhat et al., 2003). However, the role of IL-10 is controversial. IL-10 levels in blood are not correlated with PRRSV infection status (Klinge et al., 2009). Further studies are needed to determine the relationship between IFN- γ -SC and IL-10 for the protective effects of PRRSV vaccine.

The hallmark of microscopic lung lesions caused by PRRSV is interstitial pneumonia. Hence, pathological evaluation and the detection of PRRSV antigen within interstitial pneumonia are useful parameters to determine the efficacy of PRRSV vaccines. Good correlation was also observed between the decreased PRRSV load in the serum and decreased PRRSV antigen with low microscopic lesion scores in the lungs (Han et al., 2012c). In the present study, vaccination of pigs with the type 2 PRRSV was able to reduce the severity of type 1 PRRSV-induced lung lesions and the amount of type 1 PRRSV antigen within the interstitial pneumonia.

Selection of a proper challenge virus is critical when evaluating a PRRSV vaccine. The type 1 challenge virus (SNUVR090485) was isolated from pigs in 2009 and is not a vaccine virus. The two commercial type 1 PRRSV modified live vaccines were introduced in Korea in 2014. The type 1 challenge virus is a pan-European subtype 1 virus, as are most field viruses in Korea (Kim et al., 2010; Lee et al., 2010), but more pathogenic than any other Korean type 1 PRRSV isolates (Han et al., 2012c, 2013a,b).

To the best of our knowledge, this is the first experimental challenge study to evaluate this new type 2 PRRSV vaccine against heterologous type 1 PRRSV challenge. This type 2 PRRSV vaccine shows more amount of PRRSV reduction in blood than nasal swabs because IFN- γ -SC may have easier access and therefore more easily reduce PRRSV levels in the blood compared to extravasated nasal tissues. Partial cross-protection of type 2 PRRSV vaccine against type 1 PRRSV challenge provides swine practitioners and producers with clinically meaningful information because type 1 PRRSV infection is prevalent (29.4%; 20/68 swine herds) in Korea (Lee et al., 2010). Despite of partial cross-protection, these results should be interpreted carefully because this study has been conducted with only one type 1 PRRSV subtype strain. Variation in the virulence of different type 1 PRRSV field isolates in pigs has been documented (Han et al., 2013a). Moreover, a highly pathogenic type 1 PRRSV subtype 3, known as Lena strain, caused more severe respiratory disorders in young pigs than any other type 1 PRRSV subtypes 1 and 2 strains (Karniychuk et al., 2010). Future work is needed to determine the cross-protection of this type 2 PRRSV vaccine against various type 1 PRRSV field strains.

5. Conclusion

A new type 2 PRRSV modified live vaccine provides partial cross-protection against heterologous type 1 PRRSV challenge in growing pigs. Vaccinated challenged pigs showed significantly ($P < 0.05$) lower respiratory scores, viremia, macroscopic and microscopic lung lesion scores, and PRRSV-antigen with interstitial pneumonia than unvaccinated challenged pigs.

Competing interests

None.

Acknowledgements

The author's research was supported by Bio-industry Technology Development Program (Nr. 312027-3) Ministry of Agriculture, Food and Rural Affairs, Republic of Korea. This research was supported by the Zoetis in APAC. This research was also supported by contract research funds (550-20120102) of the Research Institute for Veterinary Science (RIVS) from the College of Veterinary Medicine and by the BK 21 Plus Program for Creative Veterinary Science Research. Authors thank Zoetis Korea personnel; Dr. Su-Jin Park and Dr. Soo-Hwan Kim.

References

- Allende, R., Lewis, T.L., Lu, Z., Rock, D.L., Kutish, G.F., Ali, A., Doster, 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.
- Bautista, E.M., Molitor, T.W., 1999. IFN gamma inhibits porcine reproductive and respiratory syndrome virus replication in macrophages. *Arch. Virol.* 144, 1191–1200.
- Beaver, B.V., Reed, W., Leary, S., McKiernan, B., Bain, F., Schultz, R., Bennet, B.T., Pascoe, P., Shull, E., Cork, L.C., Francis-Loyd, 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.
- Chen, N., Cao, Z., Yu, X., Deng, X., Zhao, T., Wang, L., Liu, Q., Li, X., Tian, K., 2011. Emergence of novel European genotype porcine reproductive and respiratory syndrome virus in mainland China. *J. Gen. Virol.* 92, 880–892.
- Cheon, D.-S., Chae, C., 2000. Antigenic variation and genotype of isolates of porcine reproductive and respiratory syndrome virus in Korea. *Vet. Rec.* 147, 215–218.
- Chung, W.B., Chan, W.H., Chaung, H.C., Lien, Y., Wu, C.C., Huang, Y.L., 2005. Real-time PCR for quantitation of porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 in naturally-infected and challenged pigs. *J. Virol. Methods* 124, 11–19.
- Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.C., Shaw, D.P., Goyal, S.M., McCullough, S., Morrison, R.B., Joo, H.S., Gorcyca, D., Chladek, D., 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North American and experimental reproduction of the disease in gnotobiotic pigs. *J. Vet. Diagn. Invest.* 4, 117–126.
- Conti, P., Kempuraj, D., Kandere, K., Di Gioacchino, M., Barbacane, R.C., Castellani, M.L., Felaco, M., Boucher, W., Letourneau, R., Theoharides, T.C., 2003. IL-10, an inflammatory/inhibitory cytokine, but not always. *Immunol. Lett.* 86, 123–129.
- Diaz, I., Mateu, E., 2005. Use of ELISPOT and ELISA to evaluate IFN-gamma, IL-10 and IL-4 responses in conventional pigs. *Vet. Immunol. Immunopathol.* 106, 107–112.
- 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., Chae, C., 2012a. Effects of North American porcine reproductive and respiratory syndrome virus (PRRSV)-based modified live vaccines on preimmunized sows artificially inseminated with European PRRSV-spiked semen. *Clin. Vaccine Immunol.* 19, 319–324.
- Han, K., Seo, H.W., Oh, Y., Kang, I., Park, C., Kang, S.-H., Lee, B.H., Kwon, B., Chae, C., 2012b. 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., Oh, Y., Kang, I., Park, C., Chae, C., 2012c. Pathogenesis of Korean type 1 (European genotype) porcine reproductive and respiratory syndrome virus in experimentally infected pigs. *J. Comp. Pathol.* 147, 275–284.
- Han, K., Seo, H.W., Park, C., Kang, I., Han, H.J., Kim, S.-H., Chae, C., 2013a. Comparative pathogenicity of three Korean and one Lelystad type 1 porcine reproductive and respiratory syndrome virus (pan-European subtype 1) isolates in experimentally infected pigs. *J. Comp. Pathol.* 149, 331–340.
- Han, K., Seo, H.W., Oh, Y., Kang, I., Park, C., Chae, C., 2013b. Comparison of the virulence of European and North American genotypes of porcine reproductive and respiratory syndrome virus in experimentally infected pigs. *Vet. J.* 195, 313–318.
- 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.
- Kapur, V., Elam, M.R., Pawlovich, T.M., Murtaugh, M.P., 1996. Genetic variation in porcine reproductive and respiratory syndrome virus isolates in the midwestern United States. *J. Gen. Virol.* 77, 1271–1276.
- Karniyachuk, U.U., Geldhof, M., Vanhee, M., Doorselaere, J.V., Saveleva, T.A., Nauwynck, H.J., 2010. Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate. *BMC Vet. Res.* 6, 30–39.
- Kim, S.-H., Roh, I.-S., Choi, E.-J., Lee, C., Lee, C.-H., Lee, K.-H., Lee, K.-K., Song, Y.-K., Lee, O.-S., Park, C.-K., 2010. A molecular analysis of European porcine reproductive and respiratory syndrome virus isolated in South Korea. *Vet. Microbiol.* 143, 394–400.
- Klinge, K.L., Vaughn, E.M., Roof, M.B., Bautista, E.M., Murtaugh, M.P., 2009. Age-dependent resistance to porcine reproductive and respiratory syndrome virus replication in swine. *Virol. J.* 6, 177–187.
- Kvisgaard, L.K., Hjulsgaard, C.K., Kristensen, C.S., Lauritsen, K.T., Larsen, L.E., 2013. Genetic and antigenic characterization of complete genomes of type 1 porcine reproductive and respiratory syndrome viruses (PRRSV) isolated in Denmark over a period of 10 years. *Virus Res.* 178, 197–205.
- Kwon, C.H., Kwon, B.J., Lee, H.J., Cho, J.J., Hwang, E.K., Shin, J.H., Yoon, Y.-D., Kang, Y.-B., An, S.-H., Kim, Y.-H., Huh, W., Jun, M.-H., 1994. Isolation of porcine reproductive and respiratory syndrome virus (PRRSV) in Korea. *Korean J. Vet. Res.* 34, 77–83.
- Labarque, G.G., Nauwynck, H.J., van Woensel, P.A.M., Visser, N., Pensaert, M.B., 2000. Efficacy of an American and a European serotype PRRSV vaccine after challenge with American and European wild-type strains of the virus. *Vet. Res.* 31, 97.
- Labarque, G., Van Gucht, S., Van Reeth, K., Nauwynck, H., Pensaert, M., 2003. Respiratory tract protection upon challenge of pigs vaccinated with attenuated porcine reproductive and respiratory syndrome virus vaccines. *Vet. Microbiol.* 95, 187–197.
- Labarque, G., Van Reeth, K., Nauwynck, H., Drexler, C., Van Gucht, S., Pensaert, M., 2004. Impact of genetic diversity of European-type porcine reproductive and respiratory syndrome virus strains on vaccine efficacy. *Vaccine* 22, 4183–4190.
- Lee, C., Kim, H., Kang, B., Yeom, M., Han, S., Park, S., Kim, H., Song, D., Park, B., 2010. Prevalence and phylogenetic analysis of the isolated type 1 porcine reproductive and respiratory syndrome virus from 2007 to 2008 in Korea. *Virus Genes* 40, 225–230.
- 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.
- Murtaugh, M.P., Stadejek, T., Abrahante, J.E., Lam, T.T., Leung, F.C., 2010. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus. *Virus Res.* 154, 18–30.
- Nam, E., Park, C.-K., Kim, S.-H., Joo, Y.-S., Yeo, S.-G., Lee, C., 2009. Complete genomic characterization of a European type 1 porcine reproductive and respiratory syndrome virus isolate in Korea. *Arch. Virol.* 154, 629–638.
- Nelson, E.A., Christopher-Hennings, J., Drew, T., Wensvoort, G., Collins, J.E., Benfield, D.A., 1993. Differentiation of U.S. and European isolates of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. *J. Clin. Microbiol.* 31, 3184–3189.
- 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.
- Rowland, R.R., Robinson, B., Stefanick, J., Kim, T.S., Guanghua, L., Lawson, S.R., Benfield, D.A., 2001. Inhibition of porcine reproductive and respiratory syndrome virus by interferon-gamma and recovery of virus replication with 2-aminopurine. *Arch. Virol.* 146, 539–555.
- 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, 8011–8700.
- Snijder, E.J., Meulenbergh, J.J.M., 1998. The molecular biology of arteri-viruses. *J. Gen. Virol.* 79, 961–979.
- Stadejek, T., Oleksiewicz, M.B., Scherbakov, A.V., Timina, A.M., Krabbe, J.S., Chabros, K., Potapchuk, D., 2008. Definition of subtypes in the European genotype of porcine reproductive and respiratory syndrome virus: nucleocapsid characteristics and geographical distribution in Europe. *Arch. Virol.* 153, 1479–1488.
- Suradhat, S., Thanawongnuwech, R., Poovorawan, Y., 2003. Upregulation of IL-10 gene expression in porcine peripheral mononuclear cells by porcine reproductive and respiratory syndrome virus. *J. Gen. Virol.* 84, 453–459.
- Thanawongnuwech, R., Amonsin, A., Tatsanakit, A., Damrongwatanapokin, S., 2004. Genetic and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand. *Vet. Microbiol.* 101, 9–21.
- van Woensel, P.A.M., Leifkens, K., Demaret, S., 1998. Effect on viraemia of an American and a European serotype PRRSV vaccine after challenge with European wild-type strains of the virus. *Vet. Rec.* 142, 510–512.
- 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.
- Wensvoort, G., Terpstra, C., Pol, J.M.A., ter Laak, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., van Buiten, L., den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen, P.L.J.M., Zetstra, T., de Boer, E.A., Tibben, H.J., de Jong, M.F., van't Veld, P., Greenland, G.J.R., van Gennep, J.A., Voets, M.Th., Verheijden, J.H.M., Braamskamp, J., 1991. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. *Vet. Q.* 13, 121–130.
- Xiao, Z., Batista, L., Dee, S., Halbur, P., Murtaugh, M.P., 2004. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is dependent of virus load. *J. Virol.* 78, 5923–5933.
- 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.
- Zimmerman, J.J., Benfield, D.A., Dee, S.A., Murtaugh, M.P., Stadejek, T., Stenvenson, G.W., et al., 2012. Porcine reproductive and respiratory syndrome virus (porcine arterivirus). In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.), *Diseases of Swine*. 10th ed. Wiley-Blackwell, Ames, pp. 461–486.
- 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.