

**Improved growth performance by type 2 porcine reproductive
and respiratory syndrome virus (PRRSV)-based modified live
vaccine in a herd with concurrent circulation
of type 1 and type 2 PRRSV**

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Abstract

The objective of this study was to evaluate type 2 porcine reproductive and respiratory syndrome virus (PRRSV)-based modified live vaccine in a herd with concurrent circulation of type 1 and type 2 PRRSV. Type 2 PRRSV-based modified live vaccine improved average daily gain by 48.24 grams/pig/day (631.17 grams/pig/day in the vaccinated group vs 582.93 grams/pig/day in the unvaccinated group; $P < 0.05$). Pathological examination indicated that the vaccination effectively reduced microscopic lung lesions compared with the control animals. The protection of this vaccine against type 1 and type 2 PRRSV provides clinical control of co-infection with both genotypes under field conditions.

Keywords: control, porcine reproductive and respiratory syndrome, vaccine

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is still the most economically important viral pathogen which affects the global pork industry. PRRS is characterized by reproductive failure in breeding animals and respiratory distress in growing pigs (Zimmerman et al., 2012). PRRSV can be divided into two 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 (Cha et al., 2006; Shi et al., 2010). The co-circulation of PRRS viruses of both genotypes 1 and 2 is an issue in parts of the world most notably in East Asia, including Korea. Diagnostic testing has shown that co-infection with both genotypes is highly prevalent (88.1%, 37 out of 42 farms) in Korean pig farms (C. Chae, personal observation). Therefore, cross-protection of PRRS vaccine against both genotypes is clinically important to pig producers. A new type 2 PRRSV modified live vaccine (Fostera PRRS, Zoetis, Florham, NJ, USA) was introduced into the market. This PRRS vaccine can protect pigs against type 2 PRRSV based on the manufacturer's claims (www.zoetis.com), but can cross-protect against type 1 PRRSV under experimental challenge study (Park et al., 2015). However, the efficacy of this PRRS vaccine in a pig farm with co-circulation of both genotypes has yet to be determined. Hence, the objective of this study was to evaluate the efficacy of this PRRS vaccine in a pig farm with co-circulation of both genotypes under field conditions.

Materials and Methods

A clinical field trial was conducted on a 500-sow herd with one site farrow-to-finishing production system. The farm had experienced recent losses due to respiratory diseases caused by co-infection with type 1 and type 2 PRRSV in late growing and early finishing pigs at the time of the study. Type 1 and type 2 PRRSV was isolated from 64-day-old and 84-day-old pigs, respectively, prior to the beginning of this study. Type 1 PRRSV and vaccine virus (P129, GenBank no. AF494042) share 61.0% nucleotides homology for open reading frame (ORF) 5. Type 2 PRRSV and vaccine virus (P129, GenBank no. AF494042) share 85.4% nucleotides homology for ORF5. Type 1 and type 2 PRRS viruses share 59.5% nucleotide homology for ORF5.

This study used a randomized, blinded, and weight-matched, controlled clinical trial design. To minimize sow variation, four 7-day-old piglets were selected from each sow and assigned evenly to 2 groups (30 pigs per group) using the random number generation function (Excel, Microsoft Corporation, Redmond, WA, USA). At 0 day post-vaccination (dpv, 21 days of age), pigs in vaccinated group were immunized intramuscularly with 2.0 mL of the Fostera PRRS (Zoetis, Lot No. A405013B) on the right side according to the manufacturer's label claims. Pigs in the unvaccinated group were injected on the same anatomic location with 2.0 mL of phosphate buffered saline (PBS, 0.01M, pH 7.4, 2.0 ml).

The pigs in each group were randomly assigned into pens (10 pigs/pen) and housed in the same barn. They were monitored daily for physical condition and scored weekly for clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea, abdominal breathing, and death) at 0 to 91 dpv (Halbur et al., 1995). Observers were blinded to vaccination status. Mortality rate was calculated as the number of pigs that died divided by the number of pigs initially assigned to that group within batch.

Live weight of each pig in groups 1 and 2 was measured at 0 (21 days of age), 49, 91, and 147 (168 days of age) dpv. Average daily gain (ADG, grams/pig/day) was analyzed over three time periods: between 0 and 49 dpv, 49 and 91 dpv, 91 and 147 dpv. The ADG during these various production stages was calculated as the difference between the starting and final weights divided by the duration of the stage. Data from dead pigs were included in the calculation. All animal protocols were approved by the Seoul National University Institutional Animal Care and Use Committee.

Blood samples were collected at 0, 21, 49, 70, 91, and 147 dpv. The serum samples were tested using commercial PRRSV enzyme-linked immunosorbent assay (HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc., Westbrook, MA, USA). Serum samples were considered positive for anti-PRRSV antibody if the sample/positive (S/P) ratio was ≥ 0.4 , according to the manufacturer's instructions.

QIAamp RNA Mini Kit (Qiagen Inc, Valencia, CA, USA) was used to extract RNA from the serum samples. The RNA extracts were used to quantify numbers of PRRSV genomic cDNA copies by real-time PCR as previously described (Wasilk et al., 2004; Park et al., 2014). Real-time PCR for the vaccine strain was also performed to quantify PRRSV genomic RNA copy (Park et al., 2014). The number of copies of PRRSV genomic DNA per mL of serum was converted to \log_{10} for analysis. The RNA extracts in five serum samples randomly selected from real-time PCR positive for field virus in each group at 0, 21, 49, 70, 91, and 147 dpv were used to analyze sequence of ORF5 by PCR as previously described (Do et al., 2016).

Lung samples were collected for histopathology and in situ hybridization in all pigs from each group at 147 dpv (the time of slaughter). For morphometric analysis of histopathological lesion scores in lungs, eight pieces of lung tissues (two pieces 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) were collected from each pig. Microscopic lung lesions were scored blindly on a scale from 0 (normal) to 4 (severe diffuse) by two pathologists (Halbur et al., 1995). In situ hybridization for the detection and differentiation of type 1 and type 2 PRRSV nucleic acids in the lung tissues was performed and analyzed morphometrically as previously described (Halbur et al., 1996). Number of lymphoid cells positive for type 1 and type 2 PRRSV nucleic acid in lung per unit area (0.25 mm^2) was counted using the NIH Image J 1.45s

program (<http://imagej.nih.gov/ij/download.html>) (Halbur et al., 1996).

Continuous data (ADG determined by the difference between the starting and final weights divided by the duration of the stage; PRRSV RNA [\log_{10} PRRSV genomic copies per mL] determined by real-time PCR; PRRS ELISA titer; and numbers of lung positive for PRRSV nucleic acid per unit area [0.25 mm²] determined by in situ hybridization) were analyzed with a generalized linear mixed model and the Student's *t*-test for comparison between groups was used to estimate difference at each time point. Discrete data (clinical sign and lung lesion score) were analyzed by Kruskal-Wallis and Mann-Whitney tests. The Fisher's exact test was applied to evaluate mortality rate. A value of $P < 0.05$ was considered to be significant.

Results and Discussion

The mean respiratory scores were significantly lower ($P < 0.05$) in the vaccinated pigs than in the unvaccinated pigs from 70 to 91 dpv (Figure 1). The overall mortality rates were 6.6% (2/30 pigs) in the vaccinated group and 10% (3/30 pigs) in the unvaccinated group. Diagnostic results indicated that the death of the 2 pigs in the vaccinated group was primarily due to severe diarrhea with *Salmonella* species and that of the 3 pigs in the unvaccinated group was primarily due to severe pleuropneumonia with *Actinobacillus pleuropneumoniae*. The ADG was significantly higher ($P < 0.05$) in the vaccinated pigs than in the unvaccinated pigs between 91 and 147 dpv, and between 0 and 147 dpv (Table 1).

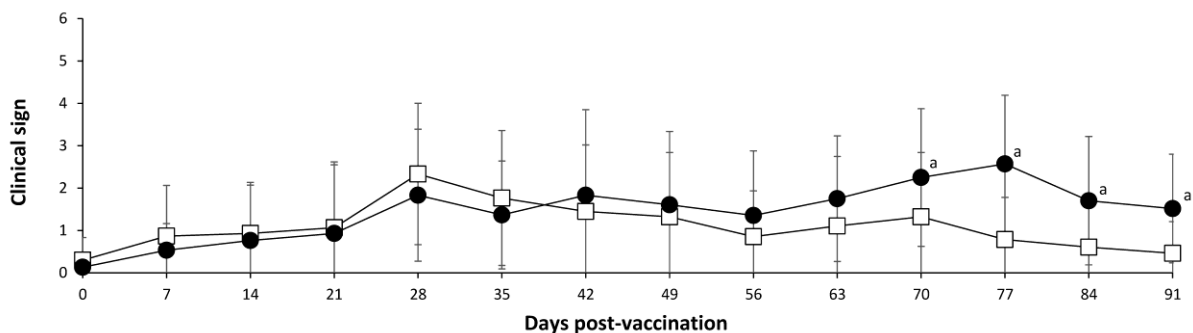


Figure 1 Mean of clinical respiratory scores of pigs from the vaccinated (■) and unvaccinated (●) groups. Variation is expressed as the standard deviation. ^aSignificant ($P < 0.05$) difference between the vaccinated and unvaccinated groups at the same day post-vaccination.

Table 1 Means (\pm standard deviation) of average daily gain (ADG), lung lesion score and numbers of type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) nucleic acid-positive cells under field conditions at different days post-vaccination (dpv)

	dpv	Vaccinated group	Unvaccinated group
ADG (g/pig/day)	0 to 49 ^a	432.58 \pm 54.16	389.43 \pm 50.40
	49 to 91	611.39 \pm 123.46	590.91 \pm 53.88
	91 to 147	808.07 \pm 72.86 ^a	753.65 \pm 58.75 ^a
	0 to 147	631.17 \pm 46.38 ^a	582.93 \pm 21.46 ^a
Microscopic lung lesion score	147	1.22 \pm 0.20 ^a	1.64 \pm 0.32 ^a
No. of type 1 PRRSV positive cells	147	0.33 \pm 0.37	0.28 \pm 0.44
No. of type 2 PRRSV Positive cells	147	1.66 \pm 0.82	2.17 \pm 1.33

^aSignificant ($P < 0.05$) difference between the vaccinated and unvaccinated groups at the same dpv

On 21 dpv, the anti-PRRSV antibody titers were significantly higher ($P < 0.05$) in the vaccinated pigs than in the unvaccinated pigs (Figure 2). The pigs from the unvaccinated group had significantly higher ($P < 0.05$) \log_{10} transformed genomic copies of type 1 PRRSV RNA in their sera at 70 dpv compared to the pigs from the vaccinated group (Figure 3A). Also, the pigs from the unvaccinated group had significantly higher ($P < 0.05$) \log_{10} transformed genomic copies of type 2 PRRSV RNA in their sera at 49 dpv compared to the pigs from the vaccinated group (Figure 3B).

The ORF5 sequences from 5 serum samples randomly selected were shown to be highly homologous (98.7-100%) to type 1 PRRS field virus and highly homologous (99.3-100%) to type 2 PRRS field virus. Vaccine virus was detected in the blood of the vaccinated pigs (Group 1) at 21 dpv (5 pigs) and 49 dpv (2 pigs). The ORF5 sequences from these serum samples in the vaccinated pigs at 21 and 49 dpv were shown to be Foster PRRS vaccine virus. No vaccine virus was detected in the blood of the unvaccinated pigs.

Lung lesion scores were significantly lower ($P < 0.05$) in the vaccinated pigs than in the unvaccinated pigs at 147 dpv (Group 2). The number of lung positive cells for type 1 and type 2 PRRSV

nucleic acid was not significantly different between the vaccinated pigs and the unvaccinated pigs at 147 dpv (Table 1).

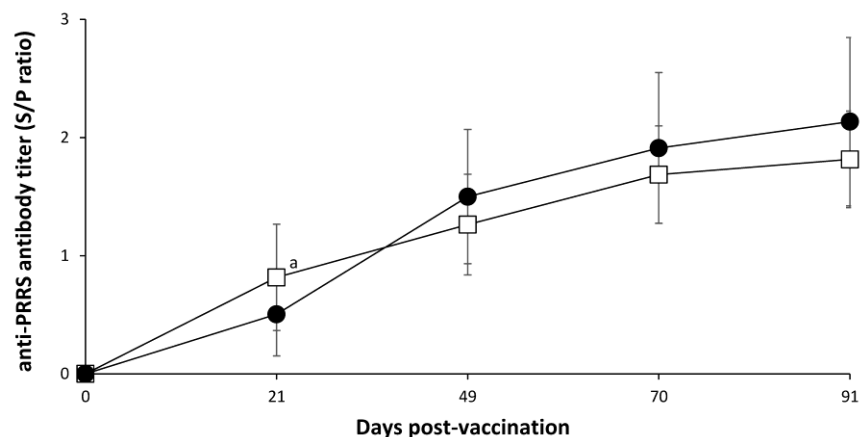


Figure 2 Mean of anti-PRRS antibody titers in serum of pigs from the vaccinated (■) and unvaccinated (●) groups. Variation is expressed as the standard deviation. ^aSignificant ($P < 0.05$) difference between the vaccinated and unvaccinated groups at the same day post-vaccination.

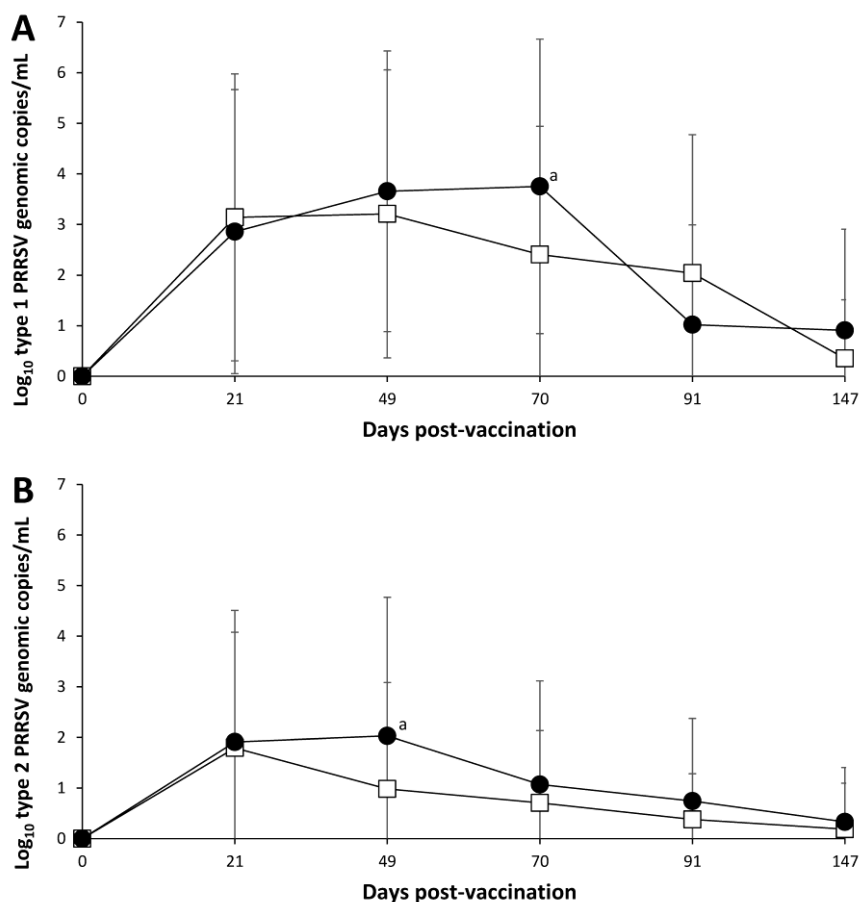


Figure 3 Mean of the log₁₀ transformed number of genomic copies of type 1 (A) and type 2 (B) PRRSV field virus RNA in serum of pigs from the vaccinated (■) and unvaccinated (●) groups. Variation is expressed as the standard deviation. ^aSignificant ($P < 0.05$) difference between the vaccinated and unvaccinated groups at the same day post-vaccination.

The results of this study demonstrate that type 2 PRRSV-based modified live vaccine improves clinical respiratory sign and growth performance of pigs raised in farms co-circulated with type 1 and type 2 PRRSV. It is often described that the infection with PRRSV increases incidence of bacterial infections, for example, with *Haemophilus parasuis*, *Streptococcus suis*,

Pasteurella multocida, or *A. pleuropneumoniae* (Zimmerman et al., 2012). Similarly, the pigs from the unvaccinated groups died of pleuropneumonia in this study. The present data are further supported by previous studies in which the same type 2 PRRSV-based modified live vaccine was efficacious in protecting growing pigs from respiratory disease

caused by heterologous type 1 and type 2 PRRSV challenge under experimental conditions (Park et al., 2014; 2015). Interestingly, the vaccinated animals showed significant reduction in type 1 and type 2 PRRSV viremia compared with the unvaccinated animals under field conditions. Similarly, vaccination of pigs with the same type 2 PRRSV-based modified live vaccine significantly reduced levels of type 1 and type 2 PRRSV viremia compared with unvaccinated pigs under experimental conditions (Park et al., 2014; 2015).

PRRS vaccination should be administered while residual maternally derived antibodies (MDA) are minimal and before pigs become naturally infected. In the present study, MDA was not detected in any pigs from the 2 groups at the time of vaccination. In this study, fifteen sows maintained low levels of anti-PRRSV antibodies, ranging from 0.5 to 0.9 S/P ratio (data not shown). Therefore, the majority of newborn piglets received low levels of colostral anti-PRRSV antibodies from their dams and might decay MDA at the time of PRRS vaccination. Both vaccinated and unvaccinated pigs might have high PRRSV ELISA antibody levels because of exposure to the circulating PRRS field virus. However, there is no evidence that high levels of PRRSV antibodies detected by ELISA play a role in protection against infection with PRRSV (Lopez and Osorio, 2014).

This study did not determine the effect of type 1 PRRSV-based vaccine on the pig farm circulated with both type 1 and type 2 PRRSV. However, a study showed that type 1 PRRSV-based modified live vaccines provided partial protection against respiratory disease caused by heterologous type 1 PRRSV challenge but no protection against heterologous type 2 PRRSV challenge in pigs during the acute phase under experimental conditions (Kim et al., 2015). There are two reasons why type 1 PRRSV-based vaccine limits the protection against type 2 PRRSV. First, type 2 PRRSV is more virulent and causes more severe respiratory disease in growing pigs than type 1 PRRSV (Halbur et al., 1996; Han et al., 2013). Second, viral loads of type 2 PRRSV in blood are relatively higher compared to type 1 PRRSV infection (Johnson et al., 2004; Han et al., 2013).

Hence, type 2 PRRSV-based modified live vaccine may provide better protection to pigs, compared to type 1 PRRSV vaccine, on farms circulated with both type 1 and type 2 PRRSV. However, when using PRRSV modified live vaccine, there is a possibility that vaccine viruses may shed. The detection of viral RNA in the serum samples may signify the shedding of vaccine viruses. Moreover, the shedding of vaccine viruses can be transmitted to naïve populations at risk such as pregnant females and regional nursery and finisher swine. Consequently, protection against type 1 and type 2 PRRSV can be provided by type 2 PRRSV-based modified live vaccine, which subsequently leads to improvement in growth performance in pig farms co-circulated with both genotypes.

Acknowledgements

This research was supported by contract research funds of the Research Institute for Veterinary Science (RIVS) from the College of Veterinary Medicine and by BK 21 PLUS Program for Creative Veterinary Science Research in the Republic of Korea. The first two authors contributed equally to this work.

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บทคัดย่อ

การเพิ่มประสิทธิภาพการเติบโตในฝูงสุกรที่ติดเชื้อ PRRSV type 1 และ type 2 ด้วยวัคซีนเชื้อเป็น type 2 Porcine reproductive and respiratory syndrome virus

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วัตถุประสงค์ของการศึกษานี้ เพื่อประเมินผลของวัคซีนเชื้อเป็นชนิด type 2 porcine reproductive and respiratory syndrome virus (PRRSV) ในฝูงสุกรที่ติดเชื้อ PRRSV ร่วม type 1 และ type 2 ผลการศึกษาพบว่า การให้วัคซีนสามารถช่วยเพิ่มอัตราการแลกเนื้อคิดเป็น 48.24 กรัมต่อสุกรต่อวัน (ในกลุ่มที่ได้รับวัคซีนมีค่า 631.17 กรัมต่อสุกรต่อวัน เมื่อเทียบกับ กลุ่มไม่ได้รับวัคซีนมีค่า 582.93 กรัมต่อสุกรต่อวัน; $P < 0.05$) และผลการศึกษาทางพยาธิวิทยาพบว่า ปอดสุกรกลุ่มที่ได้รับวัคซีน มีอัตราการของโรค PRRS น้อยกว่ากลุ่มควบคุม ดังนั้นการใช้วัคซีนชนิดนี้ในฝูงสุกรที่มีการติดเชื้อ PRRSV ร่วม type 1 และ type 2 สามารถควบคุมการแสดงอาการของโรคได้

คำสำคัญ: ควบคุม porcine reproductive and respiratory syndrome วัคซีน

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