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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Pig. 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, and 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.

QIAGEN 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 mm²) was counted using the NIH Image J 1.45s
Continuous data (ADG determined by the difference between the starting and final weights divided by the duration of the stage; PRRSV RNA \([\log_{10} \text{PRRSV genomic copies per mL}]\) determined by real-time PCR; PRRS ELISA titer; and numbers of lung positive for PRRSV nucleic acid per unit area \([0.25 \text{ mm}^2]\) 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](http://imagej.nih.gov/ij/download.html)

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

**Table 1** Means (± 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)

<table>
<thead>
<tr>
<th>dpv</th>
<th>Vaccinated group</th>
<th>Unvaccinated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG ((g/\text{pig/day}))</td>
<td>0 to 49* 432.58 ± 54.16</td>
<td>389.43 ± 50.40</td>
</tr>
<tr>
<td></td>
<td>49 to 91  611.39 ± 123.46</td>
<td>590.91 ± 53.88</td>
</tr>
<tr>
<td></td>
<td>91 to 147 808.07 ± 72.86</td>
<td>753.65 ± 58.75</td>
</tr>
<tr>
<td></td>
<td>0 to 147  631.17 ± 46.38</td>
<td>582.93 ± 21.46</td>
</tr>
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| Microscopic lung lesion score | 147 1.22 ± 0.20* | 1.64 ± 0.32* |
| No. of type 1 PRRSV positive cells | 147 0.33 ± 0.37 | 0.28 ± 0.44 |
| No. of type 2 PRRSV Positive cells | 147 1.66 ± 0.82 | 2.17 ± 1.33 |

*Significant \((P < 0.05)\) 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 Fostera 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).

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.
Comparison of the live vaccine (Fostera PRRS) against serum samples may residual maternally derived antibodies (MDA). There are two reasons why type 1 PRRSV provide better protection to pigs, if two both genotypes. Growth performance in pig farms could improve based vaccine, which subsequently leads to protection with type 2 PRRSV. Therefore, the majority of newborn piglets received low levels of colostral antibodies from their dams and might decay MDA at the time of PRRSV 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 finishing 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.

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References


บทคัดย่อ

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

อินเจ คัง1 เซ ซอก คัง2 จิยอน จอง1 ฮวัง ยอง ปาร์ค1 เชวอน คิม1 คยูฮยอน ชอย1 ซูจิน ปาร์ค1 เซฮุน คิม2 บยังฮัก คัง3 ชานฮี เช*1

วัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อประเมินผลของวัคซีนเชื้อเป็นชนิด type 2 porcine reproductive and respiratory syndrome virus (PRRSV) ในสุกรที่ติดเชื้อ PRRSV ร่วม type 1 และ type 2 ผลการศึกษาพบว่า การให้วัคซีนสามารถช่วยเพิ่มอัตราการเติบโตเนื้อคิดเป็น 48.24 กรัมต่อสุกรต่อวัน (ในกลุ่มที่ได้วัคซีนมีค่า 631.17 กรัมต่อสุกรต่อวัน เนื่องจากกลุ่มไม่ได้วัคซีนมีค่า 582.93 กรัมต่อสุกรต่อวัน; P < 0.05) และผลการศึกษาทางพยาธิวิทยาพบว่า ปอดสุกรกลุ่มที่ได้วัคซีน มีอาการของโรค PRRS น้อยกว่ากลุ่มควบคุม ดังนั้นการใช้วัคซีนชนิดนี้ในสุกรที่มีการติดเชื้อ PRRSV ร่วม type 1 และ type 2 สามารถควบคุมการแสดงอาการของโรคได้

คำสำคัญ: ควบคุม porcine reproductive and respiratory syndrome วัคซีน

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