A New Modified Live Porcine Reproductive and Respiratory Syndrome Vaccine Improves Growth Performance in Pigs under Field Conditions

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The change in growth performance resulting from a new modified live porcine reproductive and respiratory syndrome (PRRS) vaccine was evaluated under field conditions for registration with the government as guided by the Republic of Korea’s Animal and Plant Quarantine Agency. Three farms were selected based on their history of PRRS-associated respiratory diseases. On each farm, a total of 45 3-week-old pigs were randomly allocated to one of two treatment groups, (i) vaccinated (n = 25) or (ii) control (n = 20) animals. A new modified live PRRSV vaccine increased market weight by 1.26 kg/pig (104.71 kg versus 103.45 kg; P < 0.05) and decreased mortality by 17% (1.33% versus 18.33%; P < 0.05). Pathological examination indicated that vaccination effectively reduced microscopic lung lesions compared with control animals on the 3 farms. Thus, the new modified live PRRS vaccine improved growth performance and decreased mortality and lung lesions when evaluated under field conditions.

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is a small, enveloped, positive-sense, single-stranded RNA virus classified in the order Nidovirales, family Arteriviridae, genus Arterivirus (1). 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 and Asian countries (2, 3).

PRRSV infection causes late-term abortion in gilts and sows. In growing pigs, PRRSV infection is characterized by labored breathing, pronounced hyperpnea, fever, interstitial pneumonia, and increased susceptibility to bacterial diseases (4). PRRS was reported to cause economic losses of approximately 664 million dollars (U.S.) in the United States in 2012, and 55% of these economic losses was attributed to respiratory diseases in nursery and growing-finishing pigs (5).

Vaccination against PRRSV reduces the severity and frequency of respiratory signs (6, 7). Currently, PRRSV control, rather than elimination, may be a more efficient and realistic strategy due to the common use of continuous production systems with high pig density and high PRRSV prevalence in Korea. Although several options for PRRSV control are available (6), a strategic combination of vaccination and pig flow management would be beneficial for PRRSV control.

A new modified live PRRS vaccine (Fostera PRRS; Zoetis, Florham, NJ, USA) entered the international market in 2012. This vaccine is based on the virulent U.S. PRRSV isolate (P129) and is attenuated using CD163-expressing cell lines. The objective of this study was to evaluate the efficacy of this new modified live PRRS vaccine under field conditions based on clinical, virological, immunological, and pathological assays.

MATERIALS AND METHODS

Farms. The clinical field trial was conducted on 3 farms: farms A (230 sows), B (200 sows), and C (420 sows). The 3 farms are one-site and continuous-production systems. All farms were seropositive for porcine circovirus type 2 (PCV2), but clinical signs indicative of PCV2 had not been observed.

The three selected farms had suffered recent losses due to type 2 PRRSV infection and respiratory diseases in postweaning and growing pigs. At the time of the study, there were no reports of reproductive failures such as abortion, premature farrowing, stillborn, or weak-born piglets in breeding females from the three selected farms. However, outbreaks of reproductive failures had been reported in breeding females from farms A and C approximately 6 months prior to the study and from farm B approximately 8 months prior to the study.

Type 2 PRRSV was isolated in postweaning pigs from farm A (SNUVR130527, lineage 5), farm B (SNUVR130030, lineage 5), and farm C (SNUVR130081, lineage 1) based on analysis of open reading frame 5 (ORF5) (8). Vaccine strain P129 belongs to lineage 8. Phylogenetic analysis was performed for the 3 field isolates, the vaccine virus, and additional Korean field isolates (Fig. 1).

Clinical field study. All work was done in accordance with the registration guidelines of the Republic of Korea’s Animal and Plant Quarantine Agency (QIA). QIA guidelines require that 20 piglets be selected and assigned to each group of vaccinated and nonvaccinated animals. In this study, 25 piglets were assigned to the vaccination group and 20 piglets were assigned to the control group. This study used a randomized, blinded, weight- and sex-matched, controlled clinical trial design. To minimize sow variation, four to six 7-day-old piglets were randomly selected from each sow and assigned evenly to either the vaccinated or the control group. At 21 days of age, the pigs in the vaccination group (n = 25) were injected intramuscularly in the right side of the neck with 2.0 ml of the commercial modified live PRRS vaccine (Fostera PRRS; Zoetis), and control pigs (n = 20) were injected with an equal volume of phosphate-buffered saline (PBS; 0.01 M, pH 7.4, 2.0 ml) in the same anatomic location. The pigs in each group were randomly assigned by weight into pens (8 or 9 pigs per pen). The groups were housed in individual rooms within the same barn. Antibiotics (i.e., tetracycline) were given to nonvaccinated control group to help control respiratory diseases during the course of the study. Five pigs from each group were randomly selected from each farm.
FIG 1 Phylogenetic analysis. Open reading frame 5 genome from the 3 field isolates (boxed), the vaccine virus (boxed), and additional Korean field isolates are shown. An unrooted neighbor-joining tree was constructed from aligned nucleic acid sequences.

for euthanasia and necropsy at 16 weeks of age. The macroscopic lung lesions were scored at the time of slaughter for the remaining pigs. Methods used in this study were approved by the Seoul National University Institutional Animal Care and Use Committee.

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) (9). Observers were blinded to vaccination status.

Growth performance. The live weight of each pig was measured at 3, 10, and 16 weeks of age and immediately before the first batch of animals was sent to the slaughterhouse. The average daily weight gain (ADWG; grams/pig/day) was analyzed over three time periods: (i) between 3 and 10 weeks of age and the date of the first shipment to slaughter. ADWG during the different production stages was calculated as the difference between the starting and final weight divided by the duration of the stage. Data for dead or removed pigs were included in the calculation.

Mortality rate. For each group, mortality rate was calculated as the number of pigs that died divided by the number of pigs initially assigned to that group within batch. Pigs that died or were culled throughout the study were necropsied.

Serology. Blood samples from each pig were collected by jugular venipuncture at 0, 3, 14, 21, 49, 70, and 91 days postvaccination (dpv). The serum samples were tested using a commercially available PRRS enzyme-linked immunosorbent assay (ELISA; IDEXX PRRS 3X Ab test; IDEXX Laboratories Inc., Westbrook, ME, USA) and serum virus neutralization test (10, 11). Serum virus neutralization tests were performed with the virus isolated from each farm using the MARC-145 cell lines.

Quantification of PRRSV RNA in blood and nasal swabs. RNA was extracted from serum samples and nasal swabs collected at 0, 3, 14, 21, 49, 70, and 91 dpv to quantify PRRSV genomic cDNA copy numbers as previously described (12). Real-time PCR for the quantification of PRRSV RNA was designed to amplify ORF5 and ORF7 nucleotide sequences. These regions were selected because the vaccine strain (P129) and the field isolates (SNUVR130527, SNUVR130030, and SNUVR130081) shared 85.4 to 92.2% (ORF5) and 90.5 to 94.8% (ORF7) nucleotide homology at these regions. The primer sets were designed to amplify the ORF5 and ORF7 regions with primer pair 5’-CTTGACACAGTTGCTGTTGTTAC-3’ and 5’-GTTCTTCGCAAACGGCTTAATACCG-3’, respectively. The mean clinical respiratory scores were significantly (P < 0.05) lower in vaccinated animals than in control animals between 70 and 84 dpv. On farm C, the mean clinical respiratory scores were significantly (P < 0.05) lower in vaccinated animals than in control animals between 70 and 84 dpv (Fig. 2).

Growth performance. No significant difference in the ADWG was observed between vaccinated and control animals during weeks 3 to 10. However, during weeks 10 to 16, the ADWG of...
vaccinated animals was significantly (\(P < 0.05\)) higher than that of control animals on the 3 farms. The overall growth performance (from 3 weeks of age to the date of shipment of the first group of pigs to slaughter) of the vaccinated animals was significantly (\(P < 0.05\)) higher than that of the control animals at the 3 farms. The higher ADWG in vaccinated animals resulted in a 5- to 7-day-shorter time to market at the 3 farms (\(P < 0.05\)) (Table 1).

Mortality rate. The overall mortality rate was significantly (\(P < 0.05\)) lower for vaccinated animals than control animals on 2 of the 3 farms (Table 1). Respiratory signs first appeared at approximately 5 to 7 weeks of age. The pigs that died had Streptococcus suis and Pasteurella multocida isolated from their lungs. These are the most common secondary bacterial infections associated with PRRSV-induced respiratory disease.

PRRSV RNA in sera and nasal swabs. No type 2 PRRSV RNA was detected in the blood or nasal swabs from any vaccinated and control animals at 0 dpv. Genomic copies of the vaccine virus were detected in the blood (in 25/25 pigs for farm A and C and 24/25 for farm C) and nasal swabs (23/25 for farm A, 21/25 for farm B, and 22/25 for farm C) from vaccinated animals only at 3 dpv. Genomic copies of the vaccine virus were detected in the blood from vaccinated animals only at 7 (12/25 for farm A, 11/25 for farm B, and 8/25 for farm C) and 14 (2/25 for farm A, 3/25 for farm B, and 1/25 for farm C) dpv. After day 14, vaccine strain could not be detected in the blood or nasal swabs from vaccinated or control animals.

A sudden onset of viremia and nasal shedding was observed at 5 to 6 weeks of age (14 to 21 dpv) on all 3 farms. Peak levels greater than 70% positive blood and nasal samples from vaccinated and control animals were reached when the animals were 6 to 10 weeks of age (21 to 49 dpv). A decline in the number of positive samples occurred at 13 to 16 weeks of age (70 to 91 dpv). Throughout the experiment, there was no significant difference in the number of PRRSV genomic copies in the blood (Fig. 3A) or nasal swabs (Fig. 3B) of vaccinated and control animals on all 3 farms throughout the experiment. In addition, the percentages of viremic pigs and nasal shedders were not significantly different in vaccinated and control animals on all 3 farms (Table 1). No type 1 PRRSV was detected in the blood and nasal swabs throughout the experiment.

Anti-PRRSV IgG antibodies. At the time of PRRSV vaccination (3 weeks of age; 0 dpv), all pigs were negative for anti-PRRSV IgG antibodies. PRRSV-specific antibodies were detected by ELISA in the vaccinated group from 14 dpv and the control group from 21 dpv (6 weeks of age). In the control group, PRRSV-specific antibodies were determined to come from infection with a field isolate, because the vaccine virus was not detected in the serum from control animals on the 3 farms. Anti-PRRSV IgG antibodies titers were significantly (\(P < 0.05\)) higher in vaccinated group than in the control group at 14 and 21 dpv on the 3 farms (Fig. 4A).

Neutralizing antibodies. Neutralizing antibody (NA) titers were detected at 91 dpv only in vaccinated groups from the 3 farms (Fig. 4B).

Pathology. Macroscopic and microscopic lung lesions were significantly (\(P < 0.05\)) lower in vaccinated animals than in control animals at 16 weeks of age (91 dpv) on the 3 farms. Macroscopic lung lesions were significantly (\(P < 0.05\)) lower in vaccinated animals than in control animals on the date of shipment to slaughter on farm B (Table 1).
**TABLE 1**: Clinical, virological, and pathological results for vaccinated and control animals on the 3 farms.

| Parameter                          | Interval or age (wks) | Vaccinated animals | Control animals |  |
|------------------------------------|-----------------------|--------------------|----------------|---|
|                                    |                       | Farm A             | Farm B          | Farm C |
| **ADWG (g/pig/day)**               | 3–10                  | 401 ± 51           | 416 ± 58        | 454 ± 58 |
|                                    | 10–16                 | 638 ± 82*          | 648 ± 62*       | 708 ± 54* |
|                                    | 16-S                  | 797 ± 43*          | 841 ± 72*       | 878 ± 71* |
|                                    | 3-S                   | 630 ± 21*          | 647 ± 26*       | 691 ± 27* |
| **Market wt (kg)**                 |                       |                    |                |    |
|                                    | 103.548               | 102.3              | 108.2*          |      |
| **Days to market**                 |                       | 181†               | 174†            | 173† |
| **Mortality rate (no. dead/total)**|                       | 0/25†              | 1/25            | 0/25† |
| **No. of nasal shedders/total**    | 3                     | 0/25               | 0/25            | 0/25 |
|                                    | 6                     | 19/25              | 18/25           | 18/25 |
|                                    | 10                    | 8/25               | 19/24           | 11/25 |
|                                    | 16                    | 0/25               | 5/24            | 6/25 |
| **Macroscopic lung lesion score**  | 16                    | 9 ± 8.4†           | 15.2 ± 2.1†     | 5 ± 5.1† |
|                                    | 5                     | 4.2 ± 2.9          | 6 ± 4.2†        | 2.2 ± 2.6 |
| **Microscopic lung lesion score**  | 16                    | 0.4 ± 0.5†         | 0.6 ± 0.7†      | 0.5 ± 0.9† |
|                                    | 5                     | 0.2 ± 0.3          | 0.4 ± 0.3†      | 0.2 ± 0.2 |
| **PRRSV antigen score**            |                       | 1.7 ± 1.4          | 1.4 ± 1.3       | 1.1 ± 1.4 |

* S, date of shipment to slaughter. †, significantly higher value for vaccinated animals than control animals (P < 0.05); †, significantly lower value for vaccinated animals than control animals (P < 0.05).

**Immunohistochemistry.** No significant differences in mean number of PRRSV-positive cells per unit area of lung were detected between the two groups on the 3 farms throughout the experiment (Table 1).

**DISCUSSION**

A new modified live PRRS vaccine improved growth performance and reduced mortality and lung lesions under field conditions. The farms included in this field trial exhibited the typical pattern of PRRSV infection seen when nursery piglets, aged 35 to 49 days, were infected in Korean field situations. Clinical respiratory signs were also improved in vaccinated animals on all 3 farms. There is minimal peer-reviewed information on the efficacy of the PRRSV vaccines based on the type 2 genotype under field conditions (16, 17). To our knowledge, this is the first field study of the new modified live PRRS vaccine based on the type 2 genotype.

The efficacy of PRRS vaccines is usually assessed by the reduction in viremia after challenge with a virulent virus under experimental conditions (18, 19, 20). However, vaccinated animals did not show any significant reduction of PRRSV viremia compared with control animals under field conditions in previous studies (16, 17) or the present study. Similarly, no change in PRRSV viremia was observed in an experimental challenge study with another modified live PRRS vaccine based on the type 2 genotype, although vaccination did improve growth performance and reduce lung lesions (21). There is a possible explanation of why PRRSV viremia was not reduced in vaccinated animals. Under field conditions, PRRSV circulates among pigs within the herd, and the possibility of exposure and re-exposure to the virus by horizontal transmission occurs after an animal becomes infected. In the present study, vaccinated and control animals were housed in the same farm buildings at all 3 farms. Therefore, vaccinated animals were exposed to higher viral pressure than in the normal field situation, where all pigs receive the vaccine.

The most important parameter for evaluating the efficacy of PRRS vaccine under field conditions is the comparison of clinical parameters such as market weight and mortality rate between vaccinated and control animals. A new modified live PRRS vaccine increased market weight by 1.26 kg/pig (104.71 kg versus 103.45 kg; P < 0.05) and decreased mortality by 17% (1.33% versus 18.33%; P < 0.05). Improved market weight by 1.26 kg/pig increased revenue by $3.25 (U.S.; $1.00 [U.S.] = 1,063 Korean won) per pig and the 17% decrease in mortality saved $45 per pig. Hence, the total economic benefits of using the new modified live PRRS vaccine is $48.25/pig based on these two parameters. Improved growth performance and reduced mortality are clinically meaningful, because most economic losses due to PRRSV infection in herds are attributed to decreased growth performance and increased mortality rate in postweaning and growing-finishing pigs (5).

Pathological evaluation is also critical to determine the efficacy of the PRRS vaccine, because the most striking and consistent type of pathological lesions induced by PRRSV is interstitial pneumonia. Even though vaccinated animals had fewer lung lesions and...
improved growth performance compared to control animals, a reduction in macroscopic lung lesions was correlated with improved weight gain in farm B only (data not shown). The lack of correlation between reduction of lung lesions and improvement of weight gain on farms A and C may be due to the fact that lung lesions had already been resolved in finishing pigs at the time of slaughter on these farms.

Modified live PRRS vaccines have the ability to replicate in the animal after administration. Subsequent shedding of the vaccine virus allows spread from pig to pig and from pigs to the environment. In the present study, the serum of vaccinated animals remained vaccine virus positive through 14 dpv. Duration of viremia in growing pigs following vaccination was found to be shorter than in a previous study, where growing pigs were found to be viremic for as long as 63 dpv after administration of the same modified live PRRS vaccine (22). It is unknown why differences in viremia were observed between these studies. They could potentially be due to differences in host susceptibility or environment between present (i.e., field conditions) and previous (i.e., experimental conditions) studies.

Under field conditions, vaccine efficacy is affected by numerous factors, including the variable virulence of field viruses, different intervals between vaccination and natural exposure to field virus, antigenic differences between vaccine and field isolates, and viral circulation within the herds compared to well-controlled experimental conditions. Notably, the vaccine strain is less likely to be shed through nasal excretions. The results of this study demonstrate that the new modified live PRRS vaccine improved

![Graph A](image1.png)

**FIG 3** Mean values of the genomic copies of PRRSV RNA in serum (A) and nasal swabs (B). Data for vaccinated (○, farm A; ■, farm B; □, farm C) and control (○, farm A; ●, farm B; ○, farm C) animals are shown. *, P < 0.05.
growth performance and reduced mortality during the fattening period.

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