Brief Communication **Peer reviewed**

Evaluation of PRRSV vaccine efficacy following infection with PRRSV 1-7-4

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Summary

Our objective was to evaluate whether porcine reproductive and respiratory syndrome virus (PRRSV) vaccination improved mortality and morbidity following experimental infection with a PRRSV restriction fragment length polymorphism 1-7-4. Results indicated that mortality and morbidity were significantly lower for vaccinated pigs as compared to unvaccinated pigs ($P < .001$).

Keywords: swine, porcine reproductive and respiratory syndrome, vaccine, mortality, robustness

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Resumen - Evaluación de la eficacia de la vacuna contra el PRRSV después de la infección por PRRSV 1-7-4

Nuestro objetivo fue evaluar si la vacunación contra el virus del síndrome reproductivo y respiratorio del cerdo (PRRSV) mejoraba la mortalidad y la morbilidad después de una infección experimental con un PRRSV con un patrón de corte de polimorfismos de longitud de fragmento de restricción del 1-7-4. Los resultados indicaron que la mortalidad y la morbilidad fueron significativamente menores para los cerdos vacunados en comparación con los cerdos no vacunados ($P < .001$).

Résumé - Évaluation de l'efficacité du vaccin contre le virus du SRRP après une infection par le VSRRP 1-7-4

Notre objectif était d'évaluer si la vaccination contre le virus du syndrome reproducteur et respiratoire porcin (SRRP) améliorait la mortalité et la morbidité suite à une infection expérimentale avec de polymorphisme de longueur des fragments de restriction du PRRSV 1-7-4. Les résultats ont indiqué que la mortalité et la morbidité étaient significativement plus faibles pour les porcs vaccinés que pour les porcs non vaccinés ($P < .001$).

The porcine reproductive and respiratory syndrome virus (PRRSV) restriction fragment length polymorphism (RFLP) variant 1-7-4 is a highly virulent virus and common throughout the Midwestern United States.[1] Costs of the disease have been estimated to be $119 to $768/sow/year.[2] Typing an RFLP consists of digestion of viral nucleic acid with restriction endonucleases followed by gel electrophoresis, resulting in different gel banding patterns dependent on sequence differences among viruses.[3] These analyses indicate that the PRRSV RFLP 1-7-4 variant is diverse, with differences in the level of pathogenicity between variants.[4]

Commercially available PRRSV vaccines have been used within the swine industry for over 30 years. Two main categories of commercially available vaccines include modified-live virus (MLV) and killed-virus vaccines; however, killed-PRRSV vaccines have not been shown to effectively confer protection or prevent disease.[5] Therefore, the use of PRRS MLV vaccines is preferred due to their ability to reduce viremia and clinical signs.[5]

One limitation that affects the efficacy of PRRS MLV vaccines is the high PRRSV mutation rate.[6] The RNA-dependent RNA polymerase of PRRSV lacks 3′ proofreading ability,[7] leading to an estimated random evolution rate between 4.71×10^2 and 9.8×10^2 per synonymous site per year.[8] Therefore, it is important to determine whether commercially available PRRSV vaccines are efficacious across variants commonly found throughout the swine industry, such as PRRSV 1-7-4. Based on this approach, the objective of this study was to estimate the effect of vaccination with a commercially available PRRS MLV vaccine on mortality and morbidity rate in pigs subsequently inoculated with PRRSV 1-7-4. The study was based on the hypothesis that vaccination would improve performance and decrease mortality as compared to unvaccinated controls.

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Materials and methods

The Pipestone Applied Research Institutional Animal Care and Use Committee approved the trial protocol, mortality standards, and caretaker handling certification (PAR IACUC 1-18). A visual assessment of pigs and their environment, including verification of food and water source, was completed daily by a caretaker under the direction of the site veterinarian. The caretaker completed daily assessment using the individual pig care scoring system that classifies animal health status.[9] The system classifies pigs as A = acute sickness, B = subacute sickness, or C = severe, chronic illness. Acute sickness was defined as a pig presenting early clinical disease signs such as inappetence, fever, and lethargy. Subacute sickness was defined as moderate disease signs, including increased anorexia and lethargy relative to class A. Severe, chronic illness was defined by severe anorexia. Pigs were treated with antibiotics if classified as B or C. If deemed immobile and unable to eat or drink, the pig was euthanized. Pigs were humanely euthanized by a qualified caretaker that had been trained by the Pipestone Welfare Department and veterinarian.

Animal source, housing, and post weaning experimental design

All pigs (N = 198) were farrowed and weaned in the same commercial farm in southern Minnesota. Pigs were polymerase chain reaction (PCR) negative for influenza A virus of swine and PRRSV (PCR and enzyme-linked immunosorbent assay negative) prior to the study and had not been vaccinated previously for PRRSV. Viruses were sequenced at the open reading frame (ORF) 5 region to differentiate vaccine strain from wild-type variants. Standard protocols were used for PRRSV PCR testing. Further, pigs showing any disease signs (eg, diarrhea or swollen joints) were not included in the study. Individual pigs were uniquely identified with ear tags. Pigs were weaned at approximately 4 weeks of age and inoculated with PRRSV RFLP 1-7-4 at approximately 8 weeks of age. At weaning, 100 of the 198 pigs were randomly allocated at the pig level after balancing for sex (barrows and gilts) to the unvaccinated group and shipped to a research nursery in southwestern Minnesota. Pigs remaining at the source farm received a 2 mL dose of a commercial PRRS MLV vaccine following manufacturer's recommendations (Ingelvac PRRS ATP, Boehringer Ingelheim) at weaning. Two days prior to inoculation with PRRSV RFLP 1-7-4, the vaccinated group was shipped to the same research facility as the unvaccinated group. Upon arrival, pigs (N = 198) were sorted into pens by vaccine status, resulting in 8 pens of pigs per treatment group. Sex was balanced within pen. Each pen housed 12 to 13 pigs; when an odd number of pigs were placed in a pen, the extra pig was a barrow. Treatment groups were assigned to pens throughout the barn so as to account for within-barn effects.

PRRSV RFLP 1-7-4 inoculation

One week prior to PRRSV inoculation, oral fluid sampling was collected on all pigs for PRRSV testing. Using a $2 \times 10^{3.5}$ 50% tissue culture infective dose of PRRSV lineage 1 RFLP 1-7-4, all pigs were experimentally infected intramuscularly at 28 days post vaccination.[6] Based on a previous study[1] where this same pathogenic variant of PRRSV was used, the attending veterinarian visited the research facility weekly to assess when antibiotic intervention was necessary to treat secondary bacterial infections, specifically *Streptococcus suis* and *Glasserella parasuis*. While the decision regarding antibiotic selection was made based on culture and sensitivity data from laboratory submissions, commonly used antibiotics for these two specific agents frequently consisted of penicillin and cephalosporin products. A list of pigs showing clinical signs of morbidity was created daily. Individual pigs showing signs of morbidity were treated for the disease. When the list reached 20% of the population, mass medication was administered.

Phenotype collection

At 0, 7, and 14 days post infection (dpi), each pig was scored with a reported robustness scoring system as previously described.[10] This 5-point scoring system assigned a clinical score based on general clinical disease signs: 1 = a normal, healthy pig showing no disease signs; 2 = a pig showing early disease signs; 3 = a pig showing moderate disease signs; 4 = a pig with advanced clinical disease; or 5 = candidate for euthanasia. Individuals recording robustness scores were blinded for vaccination status. Mortalities were recorded throughout the study. This trial was terminated 4 weeks after challenge.

Statistical analysis

This facility included 200 nursery pig spaces with 16 pens, which allowed 8 pens/treatment group. Based on a sample size calculation ($\alpha = .05$, power = 80%, and SD = 0.12) this sample size allowed for detection of a difference of 0.16 in mortality between vaccinated and unvaccinated treatment groups. Data collected from 0 to 28 dpi were analyzed using a linear fixed effects model, where vaccine status (vaccinated vs unvaccinated for PRRSV) was fitted as a fixed effect with pen (n = 16) as the experimental unit. All analyses were conducted using R software (Version 1.2.1578; The R Foundation) using the lm function. Normality and homogeneity of variance assumptions were assessed with a Shapiro-Wilks test (Shapiro.test in R) and Levene's test (leveneTest in R) where appropriate. Differences between groups were expressed as least squares means computed from the lm function of R. For the mortality and robustness score data to be analyzed at the pen level, mortality and robustness scores were averaged within pen to represent a mean percent mortality, mean robustness score at 7 dpi, and mean robustness score at 14 dpi for each pen.

Results

The PRRS MLV vaccine was detected in the vaccinated group and not in the unvaccinated group prior to inoculation. Nucleic acid sequencing of the ORF 5 region of the vaccine virus and the PRRSV 1-7-4 challenge virus indicated an 87% homology between the two viruses. Greater than 20% of pigs showed clinical disease signs at 7 and 14 dpi; thus, mass treatment was administered at these time points. At 7 dpi, 1 mL of a ceftiofur antibiotic (Excede, Zoetis) was administered because recovery of *Streptococcus suis* and *Glasserella parasuis* and corresponding antibiotic susceptibility data indicated use of this product. At 14 dpi, the same antibiotic and 0.5 mL of an anti-inflammatory drug (Predef, Zoetis) were administered to reduce fever and respiratory signs associated with PRRSV and the secondary bacteria noted above.

Mean mortality rate in the barn was 13.6%. Mortality rates (SEM) were 5% (0.03) and 22% (0.03) for the vaccinated and unvaccinated groups, respectively ($P < .001$; Table 1). The greatest number of mortalities (79%) occurred 14 dpi. All pigs received a robustness score of 1 at day 0. Mean robustness score of the barn at 7 dpi was 2.86. Mean (SEM) robustness scores at 7 dpi were 2.59 (0.13) for the vaccinated group and 3.13 (0.13) for the unvaccinated group ($P = .01$; Figure 1A). Mean robustness score of the barn at 14 dpi was 2.65. Mean (SEM) robustness scores at 14 dpi were 2.04 (0.15) for the vaccinated group and 3.25 (0.15) for the

unvaccinated group ($P < .001$; Figure 1B). Variation in clinical robustness score was greater in the unvaccinated group (Figure 1).

Discussion

The objective of this study was to evaluate the efficacy a PRRS MLV vaccine following experimental infection with PRRSV RFLP 1-7-4. The study was based on the hypothesis that vaccination would improve health and lower mortality as compared to unvaccinated controls. Under the conditions of the study, unvaccinated pigs demonstrated reduced robustness at both 7 and 14 dpi along with a higher mortality rate. Although the use of vaccine significantly reduced mortality and morbidity, 5% mortality was still observed in the vaccinated group, indicating the acknowledged limitations of this approach. Further, the mean robustness scores were 2.59 and 2.04 at 7 and 14 dpi, respectively. On average, vaccinated pigs had reduced clinical signs of PRRSV infection and a lower overall mortality rate, consistent with previous studies.[11,12]

Pathogens can have a major extrinsic effect on performance, resulting in increased variation in body weight for individuals within an infected herd. Previous research[12] suggests that variation in morbidity and pathogen exposure translates to weight and performance differences. Increased variation in robustness scores at 7 and 14 dpi within the unvaccinated group was consistent with previous research.

As with all studies, this project exhibited both strengths and limitations. Strengths included the use of a representative variant of PRRSV which provided a robust challenge, along with the use of a large number (approximately 100) of pigs per group. Limitations included the use of only a single variant of PRRSV, as the PRRSV RFLP 1-7-4 type is not homogenous. Different PRRSV RFLP 1-7-4 viruses caused varying levels of pathogenicity and virulence[4] and the PRRSV RFLP 1-7-4 variant used in this study was not characterized beyond an ORF 5 sequence. However, in a previous study,[4] three of the four 1-7-4 isolates caused more severe disease than a known moderately virulent strain. The use of a different PRRSV RFLP 1-7-4 variant, or a completely unrelated variant may have resulted in different outcomes. Therefore, while the results from this experiment support the use of a commercially available PRRS MLV vaccine for the control of PRRS, it should be noted that vaccine efficacy may vary across different variants of PRRSV.

Despite variability among viruses of the PRRSV RFLP 1-7-4 type, this virus type often causes high mortality and morbidity.[1] The time to stability (TTS), defined as time needed to wean PRRSV-negative pigs consistently from a breeding herd after a PRRSV outbreak, was significantly longer for the PRRSV RFLP 1-7-4 type than other PRRSV types.[13] Vaccination of sows and gilts with a PRRS MLV vaccine decreased TTS compared to a combination of sow PRRS MLV vaccination and gilt field virus exposure.[14] This study also reported a numerically lower total loss of pigs per 1000 sows when both sows and gilts were vaccinated relative to other vaccination strategies.

Table 1: Least squares means (SEM) for percent mortality and robustness score for vaccinated* and unvaccinated pigs following PRRSV 1-7-4 challenge

	Vaccinated (n = 100)	Unvaccinated (n = 98)	*P* value†
Mortality, %	5.0 (0.03)	22.4 (0.03)	< .001
Robustness score‡ 7 dpi	2.59 (0.13)	3.13 (0.13)	.01
Robustness score‡ 14 dpi	2.04 (0.15)	3.25 (0.15)	< .01

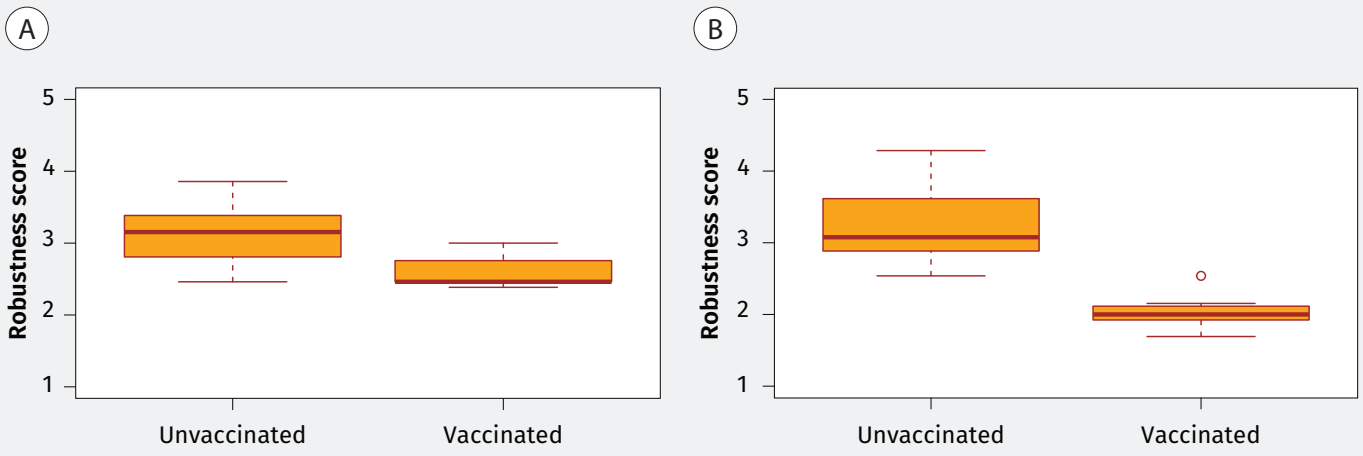
* Ingelvac PRRS ATP, Boehringer Ingelheim.

† The effect of vaccination status (vaccinated for PRRSV, or not) on each response variable using a linear model function.

‡ Robustness score, assigned on a scale from 1 to 5 where 1 = a normal, healthy pig showing no signs of disease and 5 = a candidate for euthanasia.

PRRSV = porcine reproductive and respiratory syndrome virus; dpi = days post infection.

Figure 1: Variation in robustness scores at A) 7 and B) 14 days post infection. Pigs were either vaccinated with a porcine reproductive and respiratory syndrome (PRRS) modified live virus vaccine (n = 100) or unvaccinated (n = 98) followed by challenge with PRRS virus lineage 1 isolate 1-7-4. Vaccination status had a significant effect on robustness score at both 7 ($P = .01$) and 14 days post infection ($P < .001$).



Another limitation was the inability to collect growth data from weaning to the end of the study period; however, we could hypothesize that the variation in robustness scores may have been associated with increased variation in body weight post challenge. The study aimed to test differences in mortality and morbidity between pigs vaccinated with a PRRS MLV vaccine and controls. Thus, viremia and immune response data were not collected. The PRRSV RFLP 1-7-4 is diverse and results may not be identical for other PRRSV RFLP 1-7-4 viruses. The PRRSV RFLP 1-7-4 type and a single commercially available PRRS MLV vaccine were tested. Results may not be similar for other virus types and vaccines.

In closing, results from this study demonstrated that vaccination with a PRRS MLV vaccine followed by inoculation with a highly pathogenic PRRSV strain reduced mortality rate and morbidity rate and variation in robustness scores. These results suggest that the use of commercially available PRRS MLV vaccines may be an effective tool to control clinical PRRS in the field.

Implications

Under the conditions of this study:

- An MLV vaccine reduced mortality and morbidity post PRRSV RFLP 1-7-4 infection.
- The use of commercial PRRS MLV vaccines may assist in controlling PRRS.

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Conflict of Interest

None reported.

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