Resumen: Carga viral, lesiones pulmonares y ganancia diaria promedio en un modelo de desafío del virus del síndrome reproductivo y respiratorio porcino-2

Objetivo: Determinar la viremia, el porcentaje de lesiones pulmonares, la ganancia diaria promedio (GDP) y sus asociaciones después del reto con un virus del síndrome reproductivo y respiratorio porcino-2 (PRRSV-2) linaje 1 (marco abierto 5 restriction fragment length polymorphism 1-7-4 [ORF5 RFLP 1-7-4]) en cerdos vacunados con una vacuna de virus vivo modificado (MLV) de PRRSV-2 ORF5 RFLP 1-7-4 linaje 1. La viremia se determinó a los 3, 6, y 12 días después de la exposición. Se registraron los pesos corporales para determinar la GDP durante todo el experimento. El porcentaje de lesiones pulmonares se evaluó el día 40 (12 días después de la exposición).

Resultados: La vacunación con cualquiera de las vacunas redujo (P < .001) las lesiones pulmonares, aumentó (P < .001) ADG después del desafío y controló mejor la viremia (P < .001) en comparación con los cerdos no vacunados.

Implicación: Una vacuna de PRRSV-2 de linaje 8 comercialmente disponible fue tan eficaz como una vacuna de PRRSV-2 de linaje 1 contra un desafío viral heterólogo de PRRSV-2 de linaje 1.
Porchine reproductive and respiratory syndrome virus (PRRSV)-2 is one of the most important infectious agents affecting the swine industry worldwide. It causes several forms of clinical and subclinical disease that presents with various symptoms including anorexia, fever, respiratory distress, lung lesions, abortion, general weakness, and a decrease in valuable production traits such as feed intake and average daily gain (ADG). The economic burden of the disease is due mainly to the effects of the virus in post-weaning pigs, especially through the reduction of ADG with approximately 55% of losses from PRRS occurring during the growing phase of production.

The PRRSV is a single stranded RNA virus characterized by rapid mutation rates and extensive genetic divergence. The PRRSV is classified as two species: PRRSV-1 (Betaarterivirus suid 1; formerly European PRRSV) and PRRSV-2 (Betaarterivirus suid 2; formerly North American PRRSV). The PRRSV-2 is widely spread throughout North America and Asia, and is further divided into 9 distinct lineages based on open reading frame 5 (ORF5) sequences. Over the past 20 years the lineage distribution and prevalence has varied greatly with lineage 1 being the most common strain of the virus currently in the United States. This genetic diversity is a challenge for sustained efficacy of current vaccines. For instance, a PRRSV-2 lineage 8 modified live virus (MLV) vaccine has been proven effective in protecting pigs from lung lesions and maintaining production parameters when challenged with PRRSV-2 lineages 1, 3, 5, 8, and 9.

Other studies have shown that preventative vaccination with several commercially available vaccines, including MLV vaccines, reduce lesions and other clinical signs following a PRRSV-2 challenge with similar or different lineage than that of the derived vaccine. This protective effect has been described as the ability of the vaccines to reduce lung lesions and hinder the ADG decrease that these new PRRSV-2 strains cause.

Lung lesions caused by various diseases have been associated with decreased ADG, with viral load usually correlated to the severity of the lesions. However, since some vaccines seem to show similar degrees of protection against clinical signs and lung lesions in pigs with widely divergent viremia, there is a possibility of a more direct negative relationship between PRRSV viral load and ADG. Certainly, viral load has also been negatively correlated with feed efficiency in PRRSV-infected pigs.

Our hypothesis was that lung lesions and viremia would be similar between the two vaccinated groups and that both groups would have less viremia and fewer lung lesions than nonvaccinated pigs. We also hypothesized that vaccinated animals would have similar post-vaccination shedding and would be significantly protected against a PRRSV-2 challenge, regardless of the lineage from which the MLV vaccine was derived. The objective of this study was to investigate and compare viremia in nonvaccinated pigs and in pigs vaccinated with either a lineage 8 MLV vaccine (Fostera PRRS) or a lineage 1 MLV vaccine (Prevacent PRRS) for vaccine shedding prior to challenge, and lung lesions score and viremia post challenge.

Animal care and use
The study was conducted at Swine Services Unlimited, Inc (SSUI) and was approved by the SSUI Animal Care and Use Committee.

Materials and methods
Animals
All pigs originated from a single PRRSV-naïve sow farm. Piglets on study were from litters born within 4 days of each other from second parity sows. The health status of the farm was high (ie, negative for Mycoplasma hyopneumoniae and influenza A virus, and stable for porcine circovirus type 2). Sows were vaccinated for porcine parvovirus, erysipelas, and leptospirosis (FarrowSure Gold; Zoetis) prebreeding and for rotavirus, enterotoxemia, and colibacillosis (ProSystem RCE; Merck) prior to farrowing. Piglets were given 1 mL of injectable iron (Uniferon; Pharmacosmos) the day after birth. Pigs on study were weaned at 2 weeks of age. Special nutritional care was provided for the piglets. Upon arrival at the research site, all piglets were weighed and tagged.

Experimental design
Using SAS 9.4 (Cary, NC), randomization within sex occurred by ranking pigs in descending order using their Day 0 weight. Starting with the heaviest males, each consecutive sequence of 3 animals were grouped together to form a block. The 3 pigs within each block were then randomly allocated to 1 of 3 treatment groups: 1) an experimental serial (L0817LW02; 4.36 Log_{10} TCID_{50}/2 mL) of a PRRSV lineage 8 MLV vaccine (2 mL; Fostera PRRS; Zoetis; n = 52), which was unique among commercial live PRRSV-2 vaccines in that it was attenuated by serial passing on cells expressing the porcine CD163 gene; 2) a PRRSV lineage 1 MLV vaccine (1 mL; Prevacent PRRS; Elanco; n = 50); or 3) sterile water (2 mL; n = 47). After the males were allocated, the same allocation procedure was used for females. Pigs were then placed into 1 of 3 rooms to prevent cross-contamination of vaccine virus (Figure 1). There were 8 pigs placed per pen prior to challenge. Once in their respective rooms, pigs were vaccinated according to their assigned treatment group. Pigs were observed for 15 minutes following vaccination for any adverse reactions (ie, anaphylactic shock and injection site reactions), but none were observed. One week post vaccination, individual body weight was recorded to determine if vaccination had an impact on growth.

Four cotton ropes were hung per room on a weekly basis (ie, days 7, 14, 21, and 28) and after approximately 20 minutes, fluids were collected into a plastic 50 mL conical tube. Oral fluids (n = 4/room) were shipped on wet ice on the day of collection to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) to determine vaccine shedding by quantitative polymerase chain
reaction (qPCR) for PRRSV. Four weeks after vaccination (study day 28; 6 weeks of age), all animals were challenged with PRRSV-2 ORF5 restriction fragment length polymorphism (RFLP) 1-7-4 (TCID50: 1 × 10⁴/mL dose; 2 mL intranasally and 2 mL intramuscularly; Zoetis). After the individual challenge, pigs were co-mingled within their designated block. There were 6 pigs placed per pen (2 from each treatment; 1 male and 1 female). Body weights and blood samples were taken on the day of, but prior to, challenge and co-mingling. Thereafter, blood samples to test for viremia were obtained on days 3, 6, and 12 post challenge. Blood was collected using one needle and one vacutainer tube per pig. Blood was transported in a cooler on wet ice back to the laboratory. Blood samples were processed by centrifugation for 10 minutes at 1800×g. Serum was stored at -70°C until shipped on dry ice as one shipment to ISU VDL. Twelve days post challenge (study day 40), body weights were recorded, and necropsies performed to determine percentage of lung lesions. Each individual lobe of the lungs was assessed for gross surface lesions by Dr Mueller and lesion score recorded. The calculation to determine percentage lung lesions was as follows: Percentage of total lung with lesions = 100 × (0.10 × left cranial lobe) + (0.10 × left middle lobe) + (0.25 × left caudal lobe) + (0.10 × right cranial lobe) + (0.10 × right middle lobe) + (0.25 × right caudal lobe) + (10 × accessory lobe). All serum samples from the study were shipped to ISU VDL where qPCR for PRRSV was performed. To determine potential post-vaccination shedding, PRRSV reverse transcriptase-qPCR (RT-qPCR) was performed on oral fluids. Moreover, due to positive PCR cycle threshold (Ct) values on day 28 (just prior to challenge), ORF5 genomic sequencing was completed by ISU VDL on 6 pigs (3 pigs/room) to confirm that it was vaccine virus and not wild-type virus.

qPCR analysis
All samples were sent at the conclusion of the experiment and processed by ISU VDL. Briefly, nucleic acids were extracted using a Thermo Electron KingFisher Flex automated magnetic particle processor system. The 5X MagMAX™ Pathogen RNA/DNA Kit (Applied Biosystems) was used with the Thermo Electron KingFisher Flex according to manufacturer specifications. The PRRSV RT-qPCR was performed using the 10X PRRSV Primer Probe Mix V2 from the VetMAX PRRSV NA and EU kit. The assay was modified from the original kit to use TaqMan Fast Virus 1-Step Master Mix (4×) along with the addition of Amplitaq 360 DNA Polymerase. Each reaction consisted of 6.5µL of TaqMan Fast Virus 1-Step Master Mix (4×), 0.8µL Amplitaq 360 DNA Polymerase (5U/µL), 2.7µL of nuclease-free water, 2.0µL of the 10X PRRSV Primer Probe Mix V2, and 8.0µL of nucleic acid template. The assay was run on the ABI-7500 Fast system, using the 7500 Fast System SDS Software Version 1.4.0.27. All samples were assayed within two days, with each day on separate plates. To control for plate-to-plate variation, a positive extraction control and negative extraction control were included on each extraction plate, which went through the entire process, as well as a negative amplification control that went through just the PCR step. Statistical Process Control (SPC) charting of the Cts of the positive controls were plotted to ensure Ct values were within allowed ranges. If they were not, the testing was repeated. The assay also included an internal positive control added to each sample at the time of extraction. This internal positive control needed to be detected in every sample to verify the process was performed correctly.
**Statistical analysis**

This study was conducted as a split-plot design with sex as the whole-plot factor and treatment as the split-plot factor. Individual animal was the experimental unit. Tests for normality and goodness of fit (Shapiro-Wilk test) were run for all data via PROC Univariate (SAS 9.4). Percentage lung lesions was transformed by the arcsine (square-root [%]) transformation and analyzed by a generalized linear mixed model (GLMM) approach (SAS Proc Mixed Procedure; SAS 9.4). Initial weight, day 40 weight (end of study) and ADG from day 28 (time of co-mingling) to day 40 were analyzed with a GLMM approach. For all variables, the model consisted of the fixed effects of treatment, sex, and the interaction of treatment × sex, and the random effects of room, pen (room), block (room × pen × sex) and the residual error. Body weights and ADG before co-mingling were summarized but not statistically analyzed.

For viremia, Ct values were transformed by natural log transformation prior to statistical analysis, as they were not normally distributed. Transformed values were analyzed using a GLMM approach for repeated measures. Using the PROC Mixed Procedure, transformed data were analyzed with a model that considered the fixed effects of treatment, sex, day, treatment × sex, treatment × day, sex × day, and treatment × sex × day and the random effects of room, pen (room), block (room × pen × sex) and the residual error. Day was the repeated factor. Pig was the subject. The covariance structure in the repeated measures analysis was investigated using six structural assumptions: compound symmetry, heterogeneous compound symmetry, spatial power, first order autoregressive, heterogeneous first order autoregressive, and unstructured. The assumption giving the minimum value of the Akaike’s Information Criterion was selected in the final analysis. Unstructured was the selected covariance structure. Because the treatment × day interaction was significant, LSMeans comparisons were assessed for each study day. For all variables of interest, treatment and all interactions were assessed at the 5% level.

**Results**

Prior to co-mingling, oral fluids analyzed for PRRSV demonstrated that there was potential shedding of vaccine virus in pigs from both vaccinated treatment groups (Figure 2). There was no evidence of PRRSV within the samples collected from the nonvaccinated room indicating that there was no contamination of vaccine virus to the control pigs. Moreover, weights obtained on day 7 were numerically similar across treatment groups (Table 1).

All nonvaccinated control pigs were negative for PRRSV prior to challenge, and all pigs became viremic after challenge (Figure 3). Pigs from both vaccinated treatment groups had similar viremia levels on day 28 (just prior to challenge), and sequencing data indicated that these positive values were vaccine virus, not wild-type PRRSV-2 (data not shown). At 3- and 6-days post challenge (study days 31 and 34), PRRSV-2 Ct values for pigs vaccinated with the lineage 1 vaccine were decreased (\(P < .001\) on day 3; \(P = .02\) on day 6) indicating a greater viral load compared to lineage 8 vaccinated and nonvaccinated control pigs, which did not differ (Figure 3A). By day of necropsy (day 40; 12 days post challenge), pigs vaccinated with the lineage 1 vaccine had significantly greater (\(P = .005\)) Ct values compared to pigs vaccinated with the lineage 8 vaccine, that in turn exhibited significantly greater (\(P < .001\)) Ct values compared to nonvaccinated control pigs (Figure 3A). To remove the effect of vaccine virus from the data (Figure 3A), the percentage change from time 0 (day 28) was analyzed for each pig. On day 31 and 34 (3- and 6-days post challenge), nonvaccinated pigs experienced the greatest (\(P < .001\)) decrease in Ct values (Figure 3B) from day 28. Lineage 1 MLV vaccinated pigs had a greater decrease in Ct values compared to lineage 8 vaccinated pigs on days 31 (\(P < .001\)) and 34 (\(P = .01\)). By day 40 (day of necropsy), the percentage change was significantly greater in the nonvaccinated control pigs compared to either vaccinated group (\(P < .001\)), which did not differ (Figure 3B).

On day 40 (12 days post challenge) the GMean (SEM) percentage of lung lesions in the nonvaccinated group (20.03% [19.16%]) was significantly greater than in either of the vaccinated groups (\(P < .001\)). The GMean (SEM) percentage of lung lesions in the lineage 8 vaccinated group (2.55% [0.82%]) and in the lineage 1 vaccinated group (1.60% [0.66%]) were not significantly different from each other (\(P = .36\)). There was no effect of treatment (\(P = .34\)) or treatment × sex interaction (\(P = .94\)) on initial weight for animals in any of the 3 treatment groups (Table 2). As expected, males were heavier (\(P < .001\)) than females (data not shown). On day 40, nonvaccinated pigs were lighter (\(P < .001\)) compared to the pigs in either of the vaccinated groups, which did not differ significantly from each other (Table 2). Similarly, nonvaccinated pigs had a decreased (\(P < .001\)) ADG from days 28 to 40 compared to pigs in the vaccinated treatment groups, which did not differ significantly from each other (Table 2). Statistical analyses were only performed once pigs were co-mingled (day 28). A summary of all pig weights is reported in Table 1.

**Discussion**

We fail to reject our hypothesis that viremia and lung lesions that are induced by a PRRSV-2 lineage 1 challenge are controlled by a PRRSV-2 lineage 8 vaccine, as well as a homologus vaccine (ie, a PRRSV-2 lineage 1 vaccine). Viremia (measured by qPCR) has been negatively correlated with feed efficiency in PRRSV-infected animals and negatively correlated with ADG in PCV2-infected animals.\(^{15}\) Vaccines evaluated in this study have fundamentally different methods of attenuation, but further investigations are needed to determine if this influences onset of immunity. Indeed, other studies have reported a negative relationship between lung lesions and production traits such as ADG and average daily feed intake.\(^{1,12}\) Moreover, viremia has repeatedly been negatively correlated with ADG in PRRS\(^{16}\) and other respiratory diseases.\(^{13}\)

Lung lesion scoring, viremia, and production trait measurements are the gold standards to assess protection against PRRSV.\(^{2,3,9,17}\) In this study, both vaccines were similar in their ability to protect against a PRRSV-2 lineage 1 challenge. Pigs vaccinated with either product demonstrated significant protection compared to the nonvaccinated pigs. It has been reported that vaccines derived from more contemporary viral lineages may be more protective compared to vaccines derived from older lineages.\(^{2,11,18}\) However, this study adds to the reports that a lineage 8 vaccine was just as effective as a lineage 1 vaccine at protecting against a PRRSV 1-7-4 (lineage 1) challenge. Protection against a PRRSV challenge cannot be accurately predicted by the percentage sequence identity between the virus from which the vaccine was made and the virulent PRRSV-2 in circulation.\(^{15,20}\) Strains of PRRSV are often described based on RFLP patterns, which are calculated from ORFs sequences.\(^{5,20}\) However,
Figure 2: Cycle threshold (Ct) values from oral fluid sample collected post vaccination. Upon arrival at the study site, pigs from each treatment group were placed into individual rooms and vaccinated according to their assigned treatment. Oral fluids from 4 ropes/room were collected weekly until co-mingling and challenge with porcine reproductive and respiratory syndrome virus (PRRSV) 1-7-4. Each oral fluid sample was submitted for detection of PRRSV via polymerase chain reaction. Individual samples are shown by a circle. The × indicates the average Ct value for that room at each week. Some values overlap.

RFLP designations have shortcomings as the genetic relationship between different RFLP types is not obvious, and there are many examples of two distantly related viruses sharing the same RFLP pattern. The RFLP nomenclature is most useful for distinguishing between a new virus and a limited number of resident field and vaccine viruses in a small geographic region and over a short period of time. For long term global classification of PRRSV-2, it is much more useful to use the entire ORF sequence to phylogenetically organize the genetic diversity into lineages and sublineages (or subtypes in the case of PRRSV-1). Even though many contemporary viruses are lineage 1, this does not necessarily mean they are closely related to each other or to lineage 1 vaccines. Lineage 1 is the most diverse of the PRRSV-2 lineages, and there is as much variability within lineage 1 as there is between certain other lineages. In this study, the percentage of lung lesions in nonvaccinated animals was decreased to a similar degree in animals vaccinated with either the lineage 8 vaccine or the lineage 1 vaccine. This further confirms the efficacy of the lineage 8 vaccine against PRRSV-2 lineage 1 challenges.

Our dataset adds to the scientific literature that a PRRSV-2 lineage 8 vaccine is effective against a PRRSV-2 lineage 1 viral challenge. Both vaccines proved to be similar in protecting against lung lesions, weight loss, and ADG reduction. Moreover, the lineage 8 vaccinated pigs had reduced wild-type viremia compared to lineage 1 vaccinated and nonvaccinated control animals at 3- and 6- days post challenge with an PRRSV-2 ORF5 RFLP 1-7-4 lineage 1 virus.

Implication
Under the conditions of this study:
- The PRRSV-2 lineage 8 and lineage 1 vaccines were equally effective in a PRRSV-2 lineage 1 challenge.

Acknowledgments
The authors would like to thank Dr Adam Mueller for his contribution to the animal portion of the study. Special thanks to Dr Jay Calvert for his review of the manuscript.
Table 1: Mean (SD) body weight and ADG of pigs vaccinated with a PRRSV lineage 8 vaccine, PRRSV lineage 1 vaccine, or sterile water at 2 weeks of age*

<table>
<thead>
<tr>
<th>Weight, mean (SD), kg</th>
<th>PRRSV lineage 8 vaccine (n = 52)</th>
<th>PRRSV lineage 1 vaccine (n = 50)</th>
<th>Nonvaccinated control (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>5.31 (0.99)</td>
<td>5.33 (0.88)</td>
<td>5.40 (0.93)</td>
</tr>
<tr>
<td>Day 7</td>
<td>6.98 (1.10)</td>
<td>6.86 (0.98)</td>
<td>6.92 (1.00)</td>
</tr>
<tr>
<td>Day 28</td>
<td>13.82 (2.35)</td>
<td>13.64 (1.75)</td>
<td>14.21 (2.19)</td>
</tr>
<tr>
<td>Day 40</td>
<td>18.90 (2.75)</td>
<td>19.18 (2.81)</td>
<td>16.40 (2.95)</td>
</tr>
<tr>
<td>ADG, mean (SD), kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0 to 7</td>
<td>0.24 (0.04)</td>
<td>0.22 (0.03)</td>
<td>0.22 (0.03)</td>
</tr>
<tr>
<td>Day 0 to 28</td>
<td>0.30 (0.06)</td>
<td>0.30 (0.04)</td>
<td>0.32 (0.06)</td>
</tr>
<tr>
<td>Day 0 to 40</td>
<td>0.33 (0.05)</td>
<td>0.34 (0.05)</td>
<td>0.27 (0.06)</td>
</tr>
</tbody>
</table>

* Day 0 = start of trial and vaccination; Day 7 = determine any vaccination setback; Day 28 = day of challenge and co-mingling; Day 40 = end of project.

ADG = average daily gain; PRRSV = porcine reproductive and respiratory disease virus.

**Conflict of interest**
Authors Vonnahme, Angulo, Amodie, Mellencamp, and Galina Pantoja are employed by Zoetis, Inc, the manufacturer of Fostera PRRS and have ongoing financial interest in the sale of Fostera PRRS. All authors contributed to the design of the study. Authors Vonnahme and Vasquez-Hidalgo were the primary authors; Vasquez-Hidalgo and Amodie performed the statistical analyses; all authors reviewed the manuscript prior to submission.

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**References**
Figure 3: Viremia of pigs vaccinated with either a PRRSV lineage 8 vaccine (blue), PRRSV lineage 1 vaccine (green), or sterile water (nonvaccinated; yellow) at 2 weeks of age. Challenge with PRRSV 1-7-4 occurred on day 28, and blood samples were collected on day 0 (study day 28; prior to challenge), 3-, 6-, and 12-days post challenge. A) The cycle threshold (Ct) values for wild-type PRRSV for all pigs. The positive values observed on day 0 from vaccinated pigs was determined to be their respective vaccine virus via sequencing (data not shown). B) The percentage change in Ct value was calculated to determine the proportion of Ct value that was due to the vaccine virus. LSMeans (SEM) within a day and panel with different superscripts (a,b,c) differ; \( P \leq .05 \).

<table>
<thead>
<tr>
<th>Days post challenge</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 12</th>
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<tr>
<td>Percentage change</td>
<td></td>
<td></td>
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<tr>
<td>Lineage 8 vaccine</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Lineage 1 vaccine</td>
<td>b*</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Nonvaccinated controls</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>c</td>
</tr>
</tbody>
</table>

Trt \times day: \( P < .001 \)


14. Dunkelberger JR, Boddicker NJ, Serão NVL, Young JM, Rowland RRR, Dekkers JCM. Response of pigs divergently selected for residual feed intake to experimental infection with the PRRS virus. Livest Sci. 2015;177:132-141. https://doi.org/10.1016/j.livsci.2015.01.014


<table>
<thead>
<tr>
<th></th>
<th>PRRSV lineage 8 vaccine (n = 52)</th>
<th>PRRSV lineage 1 vaccine (n = 50)</th>
<th>Nonvaccinated control (n = 47)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 0 wt, kg</td>
<td>5.37 (0.76)a</td>
<td>5.35 (0.76)a</td>
<td>5.39 (0.76)a</td>
<td>.34</td>
</tr>
<tr>
<td>D 40 wt, kg</td>
<td>18.97 (1.58)a</td>
<td>19.22 (1.58)a</td>
<td>16.49 (1.58)b</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>ADG (Day 28 to 40), kg/d</td>
<td>0.41 (0.03)a</td>
<td>0.46 (0.03)a</td>
<td>0.19 (0.03)b</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

*LSMeans (SEM) within a row with different superscripts statistically differ. ADG = average daily gain; PRRSV = porcine reproductive and respiratory syndrome virus.*


*Non-refereed references.*