Evaluating natural planned exposure protocols on rotavirus shedding patterns in gilts and the impact on their suckling pigs

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Summary
Objective: The objectives of this study were to determine the pattern of rotavirus A (RVA), rotavirus B (RVB), and rotavirus C (RVC) shedding in gilts after natural planned exposure (NPE) administration and assess the effects on piglet weaning weight, preweaning mortality, and RV shedding.

Materials and methods: A total of 70 pregnant gilts were enrolled and allocated into 4 groups. Group 1 was given NPE at 5, 4, and 3 weeks prefarrowing (WPF); Group 2 at 5 and 3 WPF; and Group 3 at 5 WPF only. Group 4 (control group) did not receive any NPE. Samples from 46 gilts and litters (5 piglets/litter) were tested at 12 sample times. Piglets were sampled weekly from 24 hours of age until 6 weeks of age and tested by quantitative reverse transcriptase-polymerase chain reaction for RVA, RVB, and RVC.

Results: There was a significant improvement in weaning weight of piglets born to gilts that received 3 NPE administrations compared to fewer or no NPE administrations. Shedding of RVA and RVB from piglets were well controlled in the farrowing room regardless of treatment group, but RVC was observed as early as 1 week of age. This study was conducted on a single farm, and the results should be carefully interpreted with knowledge of variations in farms and systems.

Implications: Three administrations of NPE to gilts prefarrowing had valuable production and economic benefits for the producer. Circulation patterns of RVA, RVB, and RVC appear to correlate; interventions for one have value against the others.

Keywords: swine, rotavirus, natural planned exposure, feedback, immunity

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Resumen - Evaluación de los protocolos de exposición natural planificada en los patrones de excreción de rotavirus en primerizas y el impacto en sus lechones

Objetivo: Los objetivos de este estudio fueron determinar el patrón de excreción del rotavirus A (RVA), rotavirus B (RVB), y el rotavirus C (RVC) en primerizas después de la administración de exposición natural planificada (NPE), y evaluar los efectos sobre el peso al destete de los lechones, mortalidad antes del destete, y la excreción del RV.

Materiales y métodos: Un total de 70 nulíparas gestantes fueron reunidas y distribuidas en 4 grupos. El grupo 1 recibió NPE a las 5, 4, y 3 semanas antes del parto (WPF); Grupo 2 a las 5 y 3 WPF; y Grupo 3 a 5 WPF solamente. El grupo 4 (grupo control) no recibió NPE. Se analizaron muestras de 46 nulíparas y sus camadas (5 lechones/camada) en 12 tiempos de muestreo. Los lechones se muestrearon semanalmente desde las 24 horas hasta las 6 semanas de edad y se analizaron mediante reacción en cadena de la polimerasa con transcriptasa inversa cuantitativa para RVA, RVB, y RVC.

Resultados: Hubo una mejora significativa en el peso al destete de los lechones nacidos de primerizas que recibieron 3 administraciones de NPE en comparación con menos o ninguna administración de NPE. La excreción de RVA y RVB de los lechones estuvo bien controlada en la sala de partos, independientemente del grupo de tratamiento, pero se observó RVC a la semana de edad. Este estudio se realizó en una sola granja por lo que los resultados deben interpretarse cuidadosamente debido a las variaciones en las granjas y los sistemas.

Implicaciones: Tres administraciones de NPE a las primerizas antes del parto tuvieron un beneficio productivo y económico para el productor. Los patrones de circulación del RVA, RVB, y RVC parecen estar correlacionados; las intervenciones para uno tienen valor frente a los otros.
X:

Objectif: Les objectifs de cette étude étaient de déterminer le schéma d'excrétion du rotavirus A (RVA), du rotavirus B (RVB), et du rotavirus C (RVC) chez les cochettes après une exposition naturelle planifiée (NPE) et d'évaluer les effets sur le poids au sevrage des porcelets, la mortalité avant le sevrage, et l'excrétion du RV.

Matériels et méthodes: Au total, 70 cochettes gestantes ont été recrutées et réparties en quatre groupes. Le groupe 1 a subi une NPE à 5, 4, et 3 semaines avant la mise bas (WPF); le groupe 2 à 5 et 3 WPF; et le groupe 3 à 5 WPF uniquement. Le groupe 4 (groupe témoin) n’a subi aucune NPE. Des échantillons de 46 cochettes et portées (5 porcelets/portée) ont été testés à 12 temps d’échantillonnage. Les porcelets ont été échantillonnés chaque semaine à partir de l’âge de 24 heures jusqu’à l’âge de 6 semaines et testés par réaction d’amplification en chaîne quantitative par la polymérase avec la transcriptase inverse pour RVA, RVB, et RVC.

Résultats: Il y a eu une amélioration significative du poids au sevrage des porcelets nés de cochettes ayant subi trois NPE par rapport à moins ou pas d’administrations de NPE. L’excrétion de RVA et de RVB des porcelets était bien maîtrisée dans la salle de mise bas quel que soit le groupe de traitement, mais le RVC a été observée dès l’âge d’une semaine. Cette étude a été menée sur une seule ferme et les résultats doivent être interprétés avec prudence en tenant compte des variations dans les fermes et les systèmes.

Implications: Trois NPE des cochettes en pré-maternité ont eu de précieux avantages économiques et de production pour le producteur. Les schémas de circulation des RVA, RVB, et RVC semblent corrélés; les interventions pour l’un sont bénéfiques envers les autres.

Rotaviruses (RVs) are common swine pathogens and significant causes of scours in pigs. Of 10 RV serogroups, rotavirus A (RVA), rotavirus B (RVB), and rotavirus C (RVC) are the main RVs infecting swine, with prevalence of 64%, 47%, and 58%, respectively.1 Rotaviruses increase preweaning mortality 3% to 20% and decrease weaning weight 0.5 to 1.0 lb (0.23-0.45 kg).2 The fastidious nature of RVB and RVC defies most control measures,3 and the inability to grow many RVs in cell culture impedes vaccine and diagnostic assay development.

Limited cross-protection both within and between RVA, RVB, and RVC strains further complicates control of RV disease.4,5 Neutralizing antibodies are generated to viral protein 4 (VP4) and viral protein 7 (VP7), which determine the P and G genotypes, respectively. They are structural proteins on the outer capsid of the virion.3 The diversity of swine RVA G and P genotypes (12 and 16, respectively) and RVC VP7 and VP4 genotypes (15 and 17, respectively) further confounds vaccine development and control.6,7 When vaccine and challenge strains share the same G genotype, protection from clinical disease and viral shedding occurs. Sharing the same P genotype leads to protection from clinical illness but not viral shedding. Without prior exposure and immunity to either VP7 or VP4, pigs will exhibit both viral shedding and clinical disease after challenge.8

Since the only commercially available swine RV vaccine in the United States (ProSystems RCE, Merck Animal Health) only contains 2 RVA serotypes, alternative control methods such as natural planned exposure (NPE) have been used to control RV infections by using live RV-infected material to generate immunity to specific RV strains circulating on a farm. The term “natural planned exposure” was chosen to convey that immunization was attempted through exposing animals to a natural material, rather than laboratory prepared vaccine, in a controlled manner. While NPE can elicit maternal immunity and passive lactogenic immunity for piglets, poor quality control could have harmful consequences. The NPE material selected from piglets in farrowing that exhibit clinical diarrhea without confirming the presence of RVs or the lack of other infectious pathogens can promote the spread of other diseases and minimize the benefit of immunization.9 A consistent supply of NPE material is challenging to maintain when RVs are effectively controlled, leading to a cyclic effect of clinical disease in the herd. When clinical disease and infectious material subsides, the herd returns to vulnerability and maternal immunity declines. Gilts that are introduced during a subsidence period likely lack adequate levels of immunity to protect their piglets. Since the survival and growth of piglets are directly correlated to colostrum intake,10 the lack of a consistent supply of NPE material can lead to a cycle of RV instability in the herd over time.

Natural planned exposure has been administered in the water, via ice cubes, manually sprayed into the mouths of sows, and added to feed as a gruel by thawing frozen RV infected material into water and feed. None of these methods have been subjected to controlled evaluation. The “master seed method” was developed to improve safety and increase efficacy of RV live virus feed-back.11 This method consists of identifying positive RV samples from the farm of interest, creating a laboratory stock or “master seed” of RV infected material using colostrum-deprived piglets, and saving the material to be used for future NPE preparation. Colostrum-deprived piglets are obtained by manually catching piglets as they are being born, and they are inoculated with the RV material. The piglets are euthanized after 18 to 24 hours and used to create an on-farm NPE stock to be used over the next several months. Diagnostic testing ensures the stock is positive for RVs and negative for relevant pathogens.

The objectives of this study were to determine the pattern of RV shedding in gilts after NPE administration and assess the effects on piglet weaning weight, preweaning mortality, and RV shedding.

Animal care and use

The gilts and pigs used in this study were cared for following Pork Quality Assurance Plus guidelines.

Materials and methods

Study design

This pilot study was conducted on an 1800-head commercial, breed-to-wean farm. In the years preceding this study, the farm had alternating periods of time without enteric challenges and with enteric clinical signs diagnosed as rotavirus. A total of 70 pregnant gilts were enrolled and allocated into 4 groups.
Group 1 was given NPE at 5, 4, and 3 weeks prefarrowing (WPF); Group 2 at 5 and 3 WPF; and Group 3 at 5 WPF only. Group 4 was a control group and did not receive any NPE administrations. Gilts were housed in pens of 5 to 6, with only gilts of their same treatment group in the same pen. Pens were initially enrolled by random selection using the randomize function on Microsoft Excel (Microsoft Corporation). At farrowing, 12 gilts from each group were enrolled for collection of shedding data based on inclusion criteria of a narrow farrowing timeframe and at least 6 liveborn piglets. Post farrowing, 2 litters were excluded due to savaging and agalactia. Forty-six litters (Group 1 = 12, Group 2 = 12, Group 3 = 11, Group4/Control = 11) were evaluated for shedding. Piglets from all contemporary litters to those tested were also included in the production data analysis. This led to a total of 59 litters (Group 1 = 15, Group 2 = 14, Group 3 = 14, Group 4/Control = 16) in the production data analysis.

Five piglets per litter were tagged and enrolled in the trial after birth. No intra-litter pig movement was allowed. Pigs were enrolled that appeared healthy and were visually similar in weight to the median pig size in the litter to avoid extreme piglet sizes.

Natural planned exposure
The NPE material was created using the master seed method and stored in an on-farm deep freezer. Due to their higher prevalence and more significant production impact, only RVA and RVC were included in the master seed NPE material. This was verified by monitoring the RVs on the farm prior to conducting the study by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). The viral strains used were collected at the farm where the gilts were born. Sequence analysis of VP4 and VP7 were performed on samples from the farm and from the stock to ensure the isolates matched. To prepare the NPE, 40 mL of master seed was added to approximately 14 L of water and mixed thoroughly with enough feed to generate approximately 100 doses of gruel. Each gruel dose was approximately 237 mL (1 cup).

Each gilt received 1 dose of NPE gruel administered 5 hours after daily feeding. Gilts were baited to their feeders using a small amount of dry feed. Once positioned in their feeding headstalls, 1 dose of NPE was measured and placed into each feeder space. Researchers remained at the pens until NPE was completely consumed. Samples of each batch of NPE gruel were reserved and tested.

Sampling
Fecal samples were collected from gilts immediately prior to NPE at 5 WPF and then twice per week until 2 WPF, after which weekly sampling occurred until weaning (-5, -4.5, -4, -3.5, -3, -2.5, -2, -1, 0, 1, 2, and 3 weeks) for a total of 12 fecal samples per gilt. Gilt fecal samples were collected by digital rectal examination and stored in individual 50-mL centrifuge tubes. Fecal swabs (BD BRL CultureSwab) were collected from piglets within 24 hours of birth and weekly until 6 weeks of age (0, 1, 2, 3, 4, 5, and 6 weeks of age) for a total of 7 fecal swabs per piglet. All pigs were weaned and transported from the sow farm to a nursery site between samples 3 and 4. All piglets were comingle at the nursery site, with no separation between treatment groups. To prevent contamination during sampling, gloves were changed between every gilt and litter of piglets.

Diagnostic testing
The NPE gruel, feces, and fecal swabs were tested by qRT-PCR for RVA, RVB, and RVC at Kansas State University Veterinary Diagnostic Laboratory. Isolation of RVA was performed on the NPE gruel to confirm live virus by blind-passaging 3 times on MA104 cells. Isolation of RVB and RVC was not attempted due to their fastidious nature. Gruel (500 µL) was incubated with 20 µL of TPCK-treated trypsin (Thermo Fisher Scientific) for 30 minutes at 37°C. Samples were then placed in 24-well plates containing confluent MA104 cells (ATCC). The plates were incubated for 1 hour at 37°C, washed with phosphate-buffered saline (PBS), and incubated for 5 to 6 days at 37°C in fresh minimum essential media (Sigma Aldrich) with 1% bovine serum albumin (Sigma Aldrich). After 2 freeze-thaw cycles, 2 additional passages on fresh MA104 cells were conducted. Samples with cytopathic effect were sent for qRT-PCR to confirm the growth of RVA. For qRT-PCR testing of gilt fecal samples, approximately 1 g of feces was added to 3 mL of PBS and centrifuged to create a fecal homogenate. Gilt fecal homogenates were pooled by 3 within their treatment group. Gilt pools testing positive by qRT-PCR were tested individually. Piglet fecal swabs were placed in 1 mL of PBS and were pooled by litter (n = 5 piglet fecal samples/pool). Cycle threshold (Ct) values less than 36 were considered positive for RV shedding. High and low viral shedding levels were determined based on a Ct value cut-off published for human RV infections to distinguish symptomatic and asymptomatic infections (Table 1).

Production data collection
Piglets were weighed 3 days prior to weaning. Additionally, the preweaning mortality rates for each treatment group were determined using the farm’s record-keeping system (PigKnows).

Data analysis
Statistical analysis on piglet weaning weight and preweaning mortality was conducted using PROC MIXED/PROC GLIMMIX (SAS v 9.4, SAS Institute Inc). A linear model was fit to explain the effect of treatment on adjusted weight. Least squares means were provided for each treatment group, and all pairwise comparisons of treatment groups were conducted using Tukey’s method was used to control for multiple comparisons. Similarly, a general linear model was fit to explain the effect of treatment on mortality. Significance was established a priori at P < .05. Adjusted weights were calculated by adding or subtracting 0.5 lb (0.23 kg) per pig for each day above or below 21 days of age at weaning, respectively.

<table>
<thead>
<tr>
<th>Viral shedding category</th>
<th>qRT-PCR Ct value range</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Ct &lt; 26</td>
</tr>
<tr>
<td>Low</td>
<td>26 ≥ Ct &lt; 36</td>
</tr>
<tr>
<td>None</td>
<td>Ct ≥ 36</td>
</tr>
</tbody>
</table>

Table 1: Levels of rotavirus shedding and corresponding quantitative real time polymerase chain reaction (qRT-PCR) cycle threshold (Ct) values used for data analysis
To investigate variables associated with low virus shedding in piglets, multiple mixed effects logistic regression models were constructed using the lme4 package in R (R Core Team). The outcomes considered were levels of RVA, RVB, or RVC viral shedding in the farrowing and nursery phases of the study (Tables 2 and 3). For each outcome, only the data points where piglets were shedding the virus of interest were considered. Thus, the model outcome was a trinary variable of either high, low, or no viral shedding based on the Ct value cut-offs (Table 1). Treatment group (4-level categorical variable; group 1, 2, 3, or 4) and shedding of other RV species (3-level categorical variable; high, low, or none) were included as fixed effects. A fixed effect in the farrowing models for the duration of gilt shedding prefarrowing (continuous variable, weeks) was incorporated as a proxy for the strength of lactogenic immunity, assuming longer viral shedding in gilts translates to more exposure and a greater immune response against RVA or RVC. This approach was adapted from porcine epidemic diarrhea virus research approaches. A fixed effect was included in the nursery phase models for the duration of piglet RVA or RVC shedding in the nursery phase (continuous variable, weeks) as a proxy for the generation of active immunity. This was included to analyze whether an increased duration of RV shedding in the farrowing room translated to a more robust active immune response and protection in the nursery phase. Previous research showed that piglets shedding RV after a virulent inoculation are better protected upon challenge. Litter was a random effect. Shedding of RVB was not detected until the nursery phase, so this model was not constructed, leaving 5 mixed effects models tested (Tables 2 and 3).

Results

Production impact
The mean 21-day adjusted piglet weaning weights for groups 1, 2, 3, and 4 (control) were 14.55, 13.42, 13.66, and 13.10 lb (6.06, 6.09, 6.20, and 5.94 kg), respectively. Group 1 (3 NPE administrations) weaning weight was significantly different than group 2 (2 NPE administrations), group 3 (1 NPE administration), and the control group. Tukey-Kramer adjusted two-sided P values for differences of least squares means for each treatment relative to the control group were <.001, .013, and <.001, respectively. This ultimately resulted in a mean weaning weight increase of 1.45 lb (0.66 kg) between group 1 and the control. No significant differences in preweaning mortality between treatment groups were identified.

Natural planned exposure
The NPE gruel samples were mixed using material that had previously tested positive by virus isolation for RVA. As determined by qRT-PCR, the NPE material fed to the sows yielded a lower Ct value for RVA than RVC (Table 4). The mean Ct value was 23.66 for RVA and 30.69 for RVC, while RVB was negative.

Gilt viral shedding
The qRT-PCR results for RVB were negative for gilts at every sampling point. Prior to the initial administration of NPE, 2 of 46 gilts were positive for RVA, while all gilts were negative for RVC (Figure 1). Based on qRT-PCR results at 4.5 WPF, the first feedback administration induced RVA shedding in 71.4% (25 of 35) of gilts with mean Ct = 30.11 while RVC was shed in only 20.0% of gilts (7 of 35; mean Ct = 32.96). At 4 WPF, the total number of RVA-shedding gilts decreased (20 of 35), but peak levels of shedding were observed in gilts that were RVA positive (mean Ct = 27.33). The number of gilts shedding RVC increased (14 of 35 gilts) at this time point, along with the level of shedding (mean Ct = 31.49). At week 3.5 after the second NPE administration for group 1, all 12 gilts in this group were shedding RVA, yet only 1 gilt was shedding RVC.

Group 1 had increased levels of shedding after the first 2 NPE administrations (collection points 4.5 and 3.5 WPF) but not after the final NPE administration (collected at 2.5 WPF). Group 2 exhibited increased shedding after both NPE administrations (4.5 and 2.5 WPF). In group 3, RVA shedding levels reached 63.6% (7 of 11) of gilts shedding after their single NPE administration (4.5 WPF) and slowly decreased over 2 weeks before they were all found to be negative at 2.5 WPF. One week prior to farrowing, only 1 gilt each was shedding RVA and RVC at low levels, both from group 2. At farrowing, RVA shedding was observed in gilts from all the treated groups (7 of 35 gilts). At 1 week post farrowing, a single gilt in each of the treated groups was positive for RVA and all gilts were negative by week 3. No RVC shedding was detected in treated gilts at the time of farrowing or at 1 week post farrowing. However, 4 control group gilts were positive for RVC at 1 week post farrowing. One control gilt was positive for RVA at 2 weeks, and 6 total gilts from groups 1 and 2 also became positive for RVC. By 3 weeks post farrowing, all gilts were negative for RVA and RVC. Overall, shedding of RVA was higher in treatment group gilts, while RVC shedding was higher in control group gilts. No apparent differences in RVA and RVC shedding were discerned between the treatment group gilts.

Piglet viral shedding
At week 1 post farrowing, 4.3% (2 of 46) litters were positive for RVA and 32.6% (15 of 46) were positive for RVC (Figure 2). Shedding of RVA in the farrowing room was rarely diagnosed in all treatment groups, with only 1 litter in group 1 and group 3 shedding RVA starting at week 1. One other litter in group 3 became RVA positive during week 3. Shedding of RVC began in week 1 and the piglet pools from control gilts contained the most positive litters (64%), while 17%, 42%, and 9% of litters were positive in groups 1, 2, and 3, respectively. At this time, 5 of the 11 (45.6%) control litters were shedding high levels (Ct < 26) of RVC, but by weeks 2 and 3, 1 litter (9.1%) and 0 litters (0.0%), respectively, had high levels of RVC shedding. No piglet litters were shedding RVC during the farrowing phase.

At the nursery (week 4), RV infections became much more prevalent regardless of the treatment group. At week 4, all litters were shedding high levels of RVA. High RVA shedding levels subsided to low levels (26 ≥ Ct < 36) in all but 1 litter from the control group at week 5 but returned at week 6 in 25% of litters in group 1, 58% of litters in group 2, and 73% of litters in groups 3 and 4. None of the litters that became RVA positive resolved their shedding during the study. Shedding of RVC first appeared at week 4 in 26 of 46 litters. The highest levels of RVC shedding were observed at week 5, while RVA shedding was subsiding. A reduction in RVC shedding was seen at week 6, but none of the litters stopped shedding the virus. Generally, litters testing positive for RVA or RVC in early farrowing tested positive for the respective RV at later sampling points. Piglet pools that were negative at 1 week of age remained negative until the animals were moved to the nursery.
### Table 2: Factors tested for association with lower RVA or RVC shedding by piglets in the farrowing room

<table>
<thead>
<tr>
<th>Possible factors</th>
<th>Low piglet RVA shedding</th>
<th>Low piglet RVC shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Piglet RVA shed level, farrowing room</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Piglet RVC shed level, farrowing room</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Duration of sow RVA shedding, prefarrow</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Duration of sow RVC shedding, prefarrow</td>
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<td>X</td>
</tr>
<tr>
<td>Litter ID†</td>
<td></td>
<td>X</td>
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</tbody>
</table>

* X indicates that the possible factor in the first column was tested for significance on the model outcome in the marked column.
† All factors were tested as fixed effects except Litter ID, which was modeled as a random effect.
RVA = rotavirus A; RVC = rotavirus C; ID = identification.

### Table 3: Factors tested for association with lower RVA, RVB, or RVC shedding by piglets in the nursery

<table>
<thead>
<tr>
<th>Possible factors</th>
<th>Low piglet RVA shedding</th>
<th>Low piglet RVB shedding</th>
<th>Low piglet RVC shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Piglet RVA shed level-nursery</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Piglet RVB shed level-nursery</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>Piglet RVC shed level-nursery</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Duration of piglet RVA shedding in the farrowing room</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of piglet RVC shedding in the farrowing room</td>
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<td></td>
<td>X</td>
</tr>
<tr>
<td>Litter ID†</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* X indicates that the possible factor in the first column was tested for significance on the model outcome in the marked column.
† All factors were tested as fixed effects except Litter ID, which was modeled as a random effect.
RVA = rotavirus A; RVB = rotavirus B; RVC = rotavirus C.

### Table 4: RVA and RVC cycle threshold values in natural planned exposure gruel mixture at each administration in weeks prefarrowing

<table>
<thead>
<tr>
<th></th>
<th>RVA (NPE Gruel) Ct</th>
<th>RVC (NPE Gruel) Ct</th>
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</thead>
<tbody>
<tr>
<td>NPE 1 (5 WPF)</td>
<td>24.42</td>
<td>32.55</td>
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<tr>
<td>NPE 2 (4 WPF)</td>
<td>22.46</td>
<td>29.32</td>
</tr>
<tr>
<td>NPE 3 (3 WPF)</td>
<td>24.15</td>
<td>30.30</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>23.66</td>
<td>30.69</td>
</tr>
</tbody>
</table>

RVA = rotavirus A; RVC = rotavirus C; Ct = cycle threshold; NPE = natural planned exposure; WPF = weeks prefarrowing.
Figure 1: Progression of A) rotavirus A (RVA) and B) rotavirus C (RVC) shedding levels over time in gilts receiving 3 (group 1), 2 (group 2), 1 (group 3), or no (group 4/control) doses of natural planned exposure. Gilts farrowed at week 0. Each horizontal bar represents one gilt and shifts up or down based on cycle threshold (Ct) value (low Ct values toward the top and high to negative Ct values at the bottom). High shedding is depicted as red, while low shedding and no shedding are shown as yellow and green, respectively. Black stars indicate time points that natural planned exposure was administered.
Figure 2: Progression of A) rotavirus A (RVA), B) rotavirus B (RVB), and C) rotavirus C (RVC) shedding levels over time in piglets based on quantitative real time polymerase chain reaction results on fecal samples pooled by litter. Litters were from gilts receiving 3 (group 1), 2 (group 2), 1 (group 3), or no (group 4/control) doses of natural planned exposure. Week 0 is farrowing, and week 4 is the first week in the nursery. Each horizontal bar represents one litter and shifts up or down based on cycle threshold (Ct) value (low Ct values toward the top and high to negative Ct values at the bottom). A gray bar indicates a missing sample. High shedding is depicted as red, while low shedding and no shedding are depicted as yellow and green, respectively.
Table 5: Odds ratios and 95% confidence intervals for statistically significant (P < .05) fixed effects of fitted models for RVA, RVB, and RVC shedding in each treatment group

<table>
<thead>
<tr>
<th>The odds of...*</th>
<th>Were...</th>
<th>For...</th>
<th>Compared to...</th>
<th>Odds ratio, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low RVC shedding-</td>
<td>86% lower</td>
<td>Group 3 litters (1 dose of NPE)</td>
<td>Group 4 litters (no NPE)</td>
<td>0.14 (0.02, 0.79)</td>
</tr>
<tr>
<td>farrowing room</td>
<td></td>
<td></td>
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<tr>
<td>Low RVA shedding-</td>
<td>630% higher</td>
<td>Litters shedding high levels of RVA in the nursery</td>
<td>Litters shedding low levels of RVA in the nursery</td>
<td>7.3 (1.55, 34.37)</td>
</tr>
<tr>
<td>nursery</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low RVB shedding-</td>
<td>329% higher</td>
<td>Litters shedding high levels of RVB in the nursery</td>
<td>Litters shedding low levels of RVB in the nursery</td>
<td>4.29 (1.20, 15.32)</td>
</tr>
<tr>
<td>nursery</td>
<td>69% lower</td>
<td>Litters shedding high levels of RVB in the nursery</td>
<td>Litters shedding low levels of RVB in the nursery</td>
<td>0.31 (0.11, 0.90)</td>
</tr>
<tr>
<td>Low RVC shedding-</td>
<td>564% higher</td>
<td>Litters shedding high levels of RVC in the nursery</td>
<td>Litters shedding low levels of RVC in the nursery</td>
<td>6.64 (1.27, 34.53)</td>
</tr>
<tr>
<td>nursery</td>
<td>75% higher</td>
<td>Each additional week of litter RVC shedding during farrowing</td>
<td>NA</td>
<td>1.75 (1.10, 2.79)</td>
</tr>
</tbody>
</table>

* Litters were from gilts receiving three (group 1), two (group 2), one (group 3), or no (group 4/control) doses of NPE. RVA = rotavirus A; RVB = rotavirus B; RVC = rotavirus C; NPE = natural planned exposure; NA = not applicable.

Mixed effects logistical modeling
Since all piglet litters became positive for one or more of RVA, RVB, or RVC during the study regardless of NPE exposure group, the researchers were interested in whether lower levels of viral shedding were associated with treatment group and whether lower viral shedding was related to other factors such as the concurrent shedding of other RV species, duration of gilt shedding prior to farrowing (as a potential proxy for lactogenic immunity), or duration of piglet shedding in the farrowing room (as a potential proxy for active immunity). Treatment group was not a significant predictor in any of the models except for RVC shedding in the farrowing room (Table 5). One administration of NPE was correlated to reduced odds of lower viral shedding compared to the control group by 86%. Specifically, it was determined that high RVC shedding was more likely in pigs from groups that received at least one administration of NPE. It was not possible to draw statistically significant conclusions about the effect of NPE administration on the reduction of viral shedding in all other models. The duration of gilt shedding prior to farrowing was not statistically associated with lower shedding in the farrowing room.

In the nursery phase, viral shedding was predominantly associated with the concurrent shedding of other RV species. Shedding of RVA and RVC were inversely related to each other, and higher RVA shedding was associated with increased odds of lower RVA shedding by 564% (Table 5). High RVA shedding was also correlated with increased odds of lower RVB shedding, but the change in odds was 329%. High RVC shedding was associated with increased odds of low RVA by 630% but reduced odds of low RVB shedding by 69%. The odds of lower RVC shedding in the nursery increased by 75% for each additional week that a litter was shedding RVC in the nursery.

Discussion
There was a significant difference in weaning weights of piglets born to gilts that received 3 NPE administrations compared to fewer or no NPE administrations, which is consistent with previous reports on the impact of rotavirus in suckling pigs.2 This suggests that 3 administrations of homologous NPE improved weight gain under the conditions of this study. This is also consistent with reports of success using NPE programs for other viruses such as transmissible gastroenteritis (TGE). In 1993, a study found that NPE for TGE virus relieved the farrowing house of all clinical signs of the disease and hypothesized that this was due to sow providing a higher level of immunity to their suckling piglets.18 A recent article specific to RVC showed that lower IgA and IgG titers in milk were related to increased incidence of clinical diarrhea and more viral shedding in piglets.19

Shedding of RVA and RVB from piglets was low in the farrowing room regardless of treatment group, but RVC was observed as early as 1 week of age. The RVC prevalence suggests insufficient antibody titers generated in the gilts, which are associated with higher rates of clinical disease in piglets.19 While RVC shedding was numerically more prevalent in the control group, the analysis did not identify treatment group as significantly associated with a reduction in viral shedding in any of the models. Infections with RVA had low prevalence and treatment did not affect RVA shedding in the farrowing room. The severity of piglet challenge was unknown for RVA. Perhaps NPE benefits may only be realized at higher burdens of environmental RV. Mixed effects logistical modeling highlighted the inverse association between RVA and RVC shedding, where low shedding of one RV was associated with high levels of the other. This contrasts with previous work that has shown RV infections are statistically associated in neonatal piglets.20 In bovine hosts, Chang and colleagues21 suggested infection with RVA may enhance RVC infections. Whether the observed peak RVA shedding followed by RVC shedding in...
the nursery indicates a similar dynamic relationship between RV species in pigs remains to be fully elucidated.

The piglets born to challenged gilts in this study were not fully protected from RV shedding. In fact, the treatment groups were not associated with a reduction of viral shedding, and all piglet litters were positive for RVA, RVB, and RVC by the end of the study. In the case of RVA, where very few infections were seen in the farrowing room, piglets may not have been sufficiently challenged by environmental RVA to induce active immunity. Passive maternal protection certainly hampers the development of active immunity in piglets, even though it is necessary for protecting piglets from preweaning viral infections.16,17 Various RV vaccine approaches have been studied at length but seldom include the basis of RV vaccine approaches have been shown to have lower levels of immunity generated after RV vaccination and shedding of other RVs.

The farm was selected to represent the most commercial sow farms in the United States. It was conducted on a gilt-only farm, while most commercial sow farms in the United States have a multiparous organization. The farm was selected to represent the most challenging case scenario since gilt litter have been shown to have lower levels of IgG in their colostrum than multiparous sows.24 The use of qRT-PCR testing means that infectivity of virus detected in feces and swabs cannot be determined. The limited knowledge on the optimal infectious dose of RVs in NPE gruel mixtures needs attention. Lastly, increased availability of serological assays may help to understand immune responses and differences in viral shedding.

Implications
Under the conditions of this study:
- Prefarrowing NPE may have production and economic benefits for producers.
- Infection with certain RVs may affect immunity and shedding of other RVs.
- On-farm NPE may be a feasible option for RV control.

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Conflict of interest
None reported.

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*Non-refereed references.