ORIGINAL RESEARCH PEER REVIEWED

Relationship between weekly porcine reproductive and respiratory syndrome virus exposure in breeding herds and subsequent viral shedding and mortality in the nursery

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Summary

Objective: Describe the relationship of weekly breeding herd status based on processing fluid (PF) testing for porcine reproductive and respiratory syndrome virus (PRRSV) RNA by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) on subsequent viral shedding and cumulative mortality during the nursery phase.

Materials and methods: Weekly cohorts (n = 121) of newborn piglets were classified into PRRSV exposure groups according to PRRSV detection in PF: low (quantification cycles [Cq] ≤ 27), medium (27 < Cq ≤ 34), high (34 < Cq ≤ 37), and negative (Cq > 37). At 6 weeks of age, oral fluids (OF) were collected from a subset of 41 cohorts, tested by qRT-PCR, and results used to classify the nursery shedding status into the same aforementioned categories. Cumulative nursery mortality was recorded for all 121 cohorts and compared between the different PRRSV exposure groups. Test agreement was assessed between PF and OF results of 41 cohorts. Moreover, the effect of 4:1 OF pooling on the probability of testing qRT-PCR-positive was evaluated.

Results: The nursery mortality for low Cq cohorts was 3.40 percentage points (range, 3.28-3.99) higher than other exposure groups. Overall, Cq values were higher in PF than in OF samples, and fair agreement ($\kappa = 0.2398$) between PF and OF was encountered. Compared to individual samples, 4:1 OF pooling resulted in 100% specificity and 76.92% sensitivity.

Implications: Weekly PF testing for PRRSV allowed for exposure group classification for each pig batch produced, which was a good predictor of subsequent cumulative nursery mortality.

Keywords: swine, porcine reproductive and respiratory syndrome virus, processing fluid, closeout performance, nursery mortality

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Resumen - Relación entre la exposición semanal al virus del síndrome reproductivo y respiratorio porcino en granjas reproductoras y la posterior eliminación viral y mortalidad en el destete

Objectivo: Describir la relación del estado semanal del hato reproductor basado en la prueba de fluidos de procesamiento (PF) para el ARN del virus del síndrome reproductivo y respiratorio porcino (PRRSV) mediante la reacción cuantitativa en cadena de la polimerasa con transcriptasa reversa (qRT-PCR) en la subsecuente eliminación viral y la mortalidad acumulada durante la fase de destete.

Materiales y métodos: Las cohortes semanales (n = 121) de lechones recién nacidos se clasificaron en grupos de exposición al PRRSV según la detección del PRRSV en PF: bajo (ciclos de cuantificación [Cq] ≤ 27), medio (27 < Cq ≤ 34), alto (34 < Cq ≤ 37), y negativo (Cq > 37). A las 6 semanas de edad, se colectaron fluidos orales (OF) de un subconjunto de 41 cohortes, analizados por qRT-PCR, y los resultados se utilizaron para clasificar el estado de eliminación en los destetes utilizando las mismas categorías antes mencionadas. La mortalidad acumulada en el destete se registró para las 121 cohortes y se comparó entre los diferentes grupos de exposición al PRRSV. En 41 cohortes se evaluó la concordancia de prueba entre los resultados de FP y OF. Además, se evaluó el efecto de la agrupación de OF 4:1 sobre la probabilidad de obtener una qRT-PCR-positiva.

Resultados: La mortalidad en el destete en las cohortes con bajo Cq fue de 3.40 puntos

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porcentuales (rango, 3.28-3.99) más alta que los otros tres grupos de exposición. En general, los valores de Cq fueron más altos en PF que en las muestras de OF, y se encontró una concordancia media ($\kappa = 0.2398$) entre PF y OF. En comparación con las muestras individuales, la combinación de OF 4:1 dio como resultado una especificidad del 100% y una sensibilidad del 76.92%.

Implicaciones: La prueba semanal de PF para PRRSV permitió la clasificación del grupo de exposición para cada lote de cerdos producidos, lo que fue un buen predictor de la posterior mortalidad en el destete.

Résumé - Relation entre l'exposition hebdomadaire au virus du syndrome reproducteur et respiratoire porcin dans des troupeaux de reproducteurs et l'excrétion virale et les mortalités subséquentes dans les pouponnières

Objectif: Décrire la relation du statut hebdomadaire d'un troupeau de reproducteurs basé sur les tests utilisant le liquide de procédures (PF) pour l'ARN du virus du syndrome reproducteur et respiratoire porcin (PRRSV) par réaction d'amplification en chaîne quantitative avec la transcriptase reverse (qRT-PCR) sur l'excrétion virale subséquente et la mortalité cumulative durant la période en pouponnière.

Matériels et méthodes: Des cohortes hebdomadaires (n = 121) de porcelets nouveaunés furent classifiés en groupes d'exposition au PRRSV selon la détection de PRRSV: bas (cycles de quantification [Cq] ≤ 27), moyen (27 < Cq ≤ 34), élevé (34 < Cq ≤ 37), et négatif (Cq > 37). À 6 semaines d'âge, des fluides oraux (OF) furent prélevés d'un sous-groupe de 41 cohortes, testés par qRT-PCR et les résultats utilisés pour classifier le statut excréteur de la pouponnière à l'intérieur des mêmes catégories que mentionnées précédemment. La mortalité cumulative dans la pouponnière fut notée pour toutes les 121 cohortes et comparée entre les différents groupes d'exposition au PRRSV. L'accord des tests fut évalué entre les résultats pour PF et OF des 41 cohortes. De plus, l'effet de regrouper les OF dans un ratio 4:1 sur la probabilité de s'avérer positif par qRT-PCR fut évalué.

Résultats: La mortalité en pouponnière pour les cohortes avec un Cq bas était de 3.40 points de pourcentage (écart, 3.28-3.99) plus élevée que dans les autres groupes d'exposition. De manière générale, les valeurs de Cq étaient plus élevées dans les échantillons de PF que dans ceux d'OF, un accord acceptable ($\kappa = 0.2398$) entre PF et OF fut observé. Comparativement aux échantillons individuels, le regroupement 4:1 a résulté en une spécificité de 100% et une sensibilité de 76.92%.

Implications: Les tests hebdomadaires sur le PF pour le PRRSV ont permis une classification en groupe d'exposition pour chaque lot de porcs produits, ce qui était un bon prédicteur de la mortalité cumulative subséquente en pouponnière.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases affecting the global swine industry. The economic losses attributed to this disease in the US swine industry was estimated to be $663.91 million annually.[1] The PRRS-attributed mortality can reach up to 20% in weaning and grower pigs.[2] Approximately 55% ($361.85 of $663.91 million) of the economic impact related to PRRS in the United States is due to production losses in the growing-pig herd.[1] Altogether, PRRS causes a loss of 9.93 million pigs per year in the United States.[1] To help the swine industry to standardize classification regarding PRRS virus (PRRSV) shedding and exposure in sow farms, a guideline was proposed in 2011 by the American Association of Swine Veterinarians (AASV).[3] This allowed veterinarians to conduct benchmarking of PRRSV status change within and between production systems.[4-6] A methodology to classify growing pigs as either positive or negative based on polymerase chain reaction and enzyme-linked immunosorbent assay test results was previously proposed.[3] However, there has been limited advancement in methodologies to classify batches of growing pigs according to PRRSV status beyond positive or negative.

Oral fluid (OF) testing was described in 2008 as a population-based specimen for PRRSV herd monitoring.[7,8] Oral fluid is a practical sample type to collect, requires less labor and time, and represents the status of more pigs in the population when compared with the use of individual serum samples.[7] Due to its usefulness to monitor PRRSV in grower animals, further evaluation[9] and guidelines for spatial sampling have been described.[10] In 2017, processing fluid (PF) was identified as a new population-based sample type to monitor PRRSV in newborn piglets.[11] Processing fluid is an aggregate population sample derived from the serosanguinous fluid recovered from piglet castration and tail docking (ie, processing), and has been shown to be a reliable, practical, and time-efficient sample type to monitor PRRSV and PRRSV shedding in the breeding herd.[12-14]

In 2018, OF and PF corresponded to 35% and 11% of all cases submitted for PRRSV RNA detection by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to the four major US swine-centric veterinary diagnostic laboratories.[15] This demonstrates a considerable use of both population-based sample types by the US swine industry for PRRSV testing. However, to the best of our knowledge, there is no data on the relationship between PRRSV qRT-PCR test results from PF samples and the subsequent nursery mortality. Also, there is no information in the peer-reviewed literature on the agreement between PRRSV qRT-PCR results on PF (typically collected at 3-5 days of age) and OF collected from the same cohort of pigs when they reach the nursery (3-7 weeks of age). Understanding these relationships will allow veterinarians to strategically design monitoring and surveillance systems to identify batches of pigs at higher risk of PRRS-attributed mortality, PRRSV shedding in the nursery, or both. Therefore, the main purpose of this study was to evaluate the relationship between PRRSV RNA qRT-PCR quantification cycle (Cq) results obtained on PF and the subsequent nursery mortality for 121 cohorts raised in field conditions. Secondary objectives were to assess the agreement of PRRSV RNA qRT-PCR results between PF (3-5 days of age) and OF (6 weeks of age) in 41 cohorts of pigs and to describe the effect of pooling OF samples (4:1) on the diagnostic sensitivity and specificity.

Materials and methods

Study design

This study was approved by the Iowa State University Institutional Animal Care and Use Committee under protocol number 3-18-8730-S. This prospective analytical

study was designed in August of 2017 and the farms were recruited between September and November 2017. The study was conducted using 2 pig flows, each with 6 commercial sow farms and 4 nursery farms between January and August 2018. The farms were geographically isolated from other production systems and were part of the same swine production system. Weekly batches of newborn piglets were monitored at sow farms for exposure to PRRSV by testing one aggregated PF sample for each cohort using a commercial qRT-PCR assay. For each cohort, qRT-PCR Cq results were categorized into PRRSV exposure groups: low, medium, high, or negative. The nursery mortality, summarized as the cohort's cumulative mortality during the nursery period (3-9 or 10 weeks of age), was recorded for each cohort. The distribution of mortality for each exposure group was recorded. Furthermore, PRRSV shedding in the nursery was assessed in 41 cohorts by testing OF samples collected at 6 weeks of age and tested for PRRSV RNA using commercial qRT-PCR-based methods. The OF sample results were categorized using the same criteria used for PF samples, based on the sample with the lowest Cq value for each cohort. The agreement criteria of PRRSV qRT-PCR results between PF and OF samples was described according to Landis and Koch criteria.[16]

Study herds and PRRSV exposure cohorts

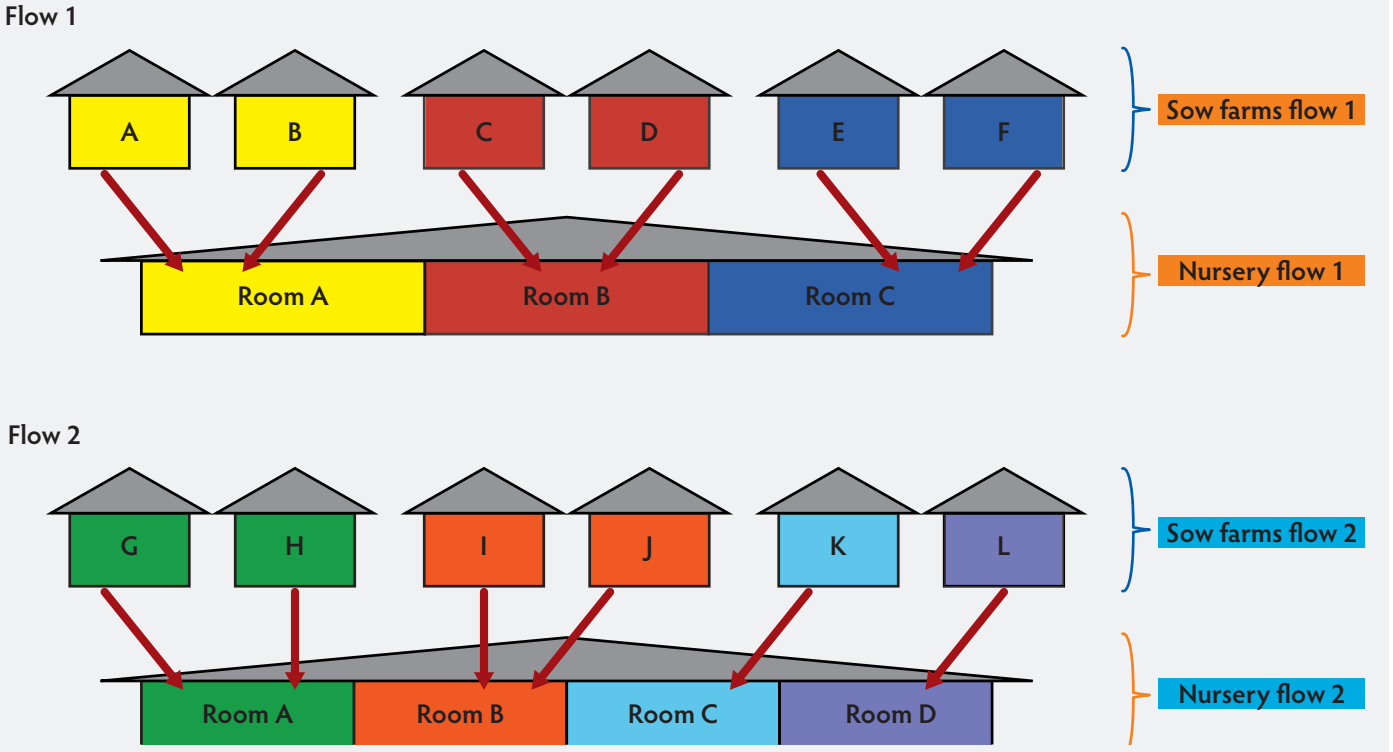
We recruited breed-to-wean herds endemically infected with PRRSV and classified as "positive unstable" according to the AASV PRRSV classification terminology.[3] All study herds reported use of PRRS Ingelvac MLV (Boehringer Ingelheim Vetmedica Inc) in the replacement gilts at 26 weeks of age. Targeting days having the highest number of processed litters, PF samples were collected 3 days per week and pooled for 1 test per cohort. A cohort was defined as a weekly group of weaned piglets (15-19 days of age) moved to one nursery barn and room. The pig flows of the study population are described in Figure 1. Eight different nursery farms (nursery farms 1-8) were used for piglet placement after weaning. Flow 1 included sow farms A to F and 4 nursery farms with 3 rooms each. Flow 2 included sow farms G to L and 4 nursery farms with 4 rooms each. In 10 (sow farms A to J) of the 12 sow farms, piglet cohorts from 2 farms were commingled in a nursery room. Piglet cohorts from the 2 largest sow farms (K and L) were not commingled and each cohort flowed into a single nursery room. For commingled rooms, piglets were placed in pens separated by sow farm of origin. The company veterinary health service standard operational procedure was to collect 4 OF samples the first week of the month from each sow farm cohort at six weeks of age. Commingled cohort PRRSV status was defined by the lowest results obtained on PF PRRSV qRT-PCR Cq values. Results of OF PRRSV qRT-PCR samples were recorded and compared to the results of PF samples of respective non-commingled flows (Figure 1).

Sample collection and diagnostic testing

For each sow farm, PF obtained from 3 days of collection within the same week were pooled into 1 weekly PF sample. The PF were stored in 50 mL Falcon tubes (Fisher Scientific), frozen at -20°C, and submitted to the Iowa State University Veterinary Diagnostic Laboratory for testing. Both PF and OF samples were tested using the same procedures for PRRSV qRT-PCR commercial kits as previously described.[17,18] The results were reported as the Cq value.[19]

During the first week of each month, OF samples were collected from cohorts (n = 41) that were six weeks of age. Non-commingled flows collected 4 OF samples and commingled flows collected 8 OF samples (4 samples per sow farm of origin) from different pens

Figure 1: Diagram demonstrating the organization of sow farm and nursery from flows 1 and 2. Of the 121 cohorts, 87 (71.9%) were commingled from sow farms A to J and 34 (28.1%) were non-commingled from sow farms K and L.



within a barn following a spatial zig-zag distribution pattern as described by Rotolo et al.[10] The OF samples were tested individually and in pools of 4:1 for PRRSV RNA by qRT-PCR.

Defining breeding herd PRRSV exposure and nursery PRRSV shedding status

The qRT-PCR test results for PF of each cohort were used to categorize PRRSV exposure of each group: low when Cq was ≤ 27, medium when 27 < Cq ≤ 34, high when 34 < Cq ≤ 37, and negative when Cq > 37. Similarly, the lowest qRT-PCR Cq value of OF samples were used to categorize the nursery PRRSV shedding status using the same cut offs established for PF (low, medium, high, and negative). The proposed Cq cutoffs for PRRSV exposure groups were based on expected 10-fold change of the amount of PRRSV RNA in the sample. Each 10-fold change in RNA copies per milliliter is mathematically proportional to 3.3 Cq values.[20] To facilitate communications regarding the level of PRRSV exposure between veterinarians and producers, the cutoffs were adjusted to the nearest integer representing the expected 10-fold change, ie, the cutoff for the medium vs high PRRSV exposure group was rounded from 33.7 to 34, and the cutoff between the low vs medium PRRSV exposure groups was rounded from 27.1 to 27.

Evaluating the effect of OF pooling on qRT-PCR testing

For the comparison between individual and pooled OF results, 66 sets of OF were tested by qRT-PCR in pools of 4:1. At the midpoint of the study, 10 PRRSV open reading frame-5 (ORF-5) sequences were performed from 6 PF and 4 OF samples. The sample having the lowest qRT-PCR Cq values were strategically selected for ORF-5 sequencing. Sequencing was performed to describe the PRRSV present in the study population.

Statistical analysis

The main objective of this study was to describe the relationship between PRRSV exposure status based on PF sample test results (low, medium, high, or negative) and the subsequent nursery mortality. This relationship was described by a generalized linear mixed model using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc), using cumulative nursery mortality counts as the dependent variable, assuming a Poisson distribution, and the exposure group as the explanatory variable in the model. The number of pigs placed in the nursery was used as the offset variable. Additionally, the mortality count difference between groups was tested by a Chi-square test. A similar procedure was used to analyze the relationship between the level of PRRSV shedding in the nursery and the cumulative nursery mortality using OF results. Agreement of categorized qRT-PCR Cq results between PF and OF were reported using crude agreement, and Cohen's Kappa test. Kappa analysis was performed in SAS 9.4. Specificity, sensitivity, positive predictive value, and negative predictive value for the OF 4:1 pooling effect compared to individual sample result were calculated.

Results

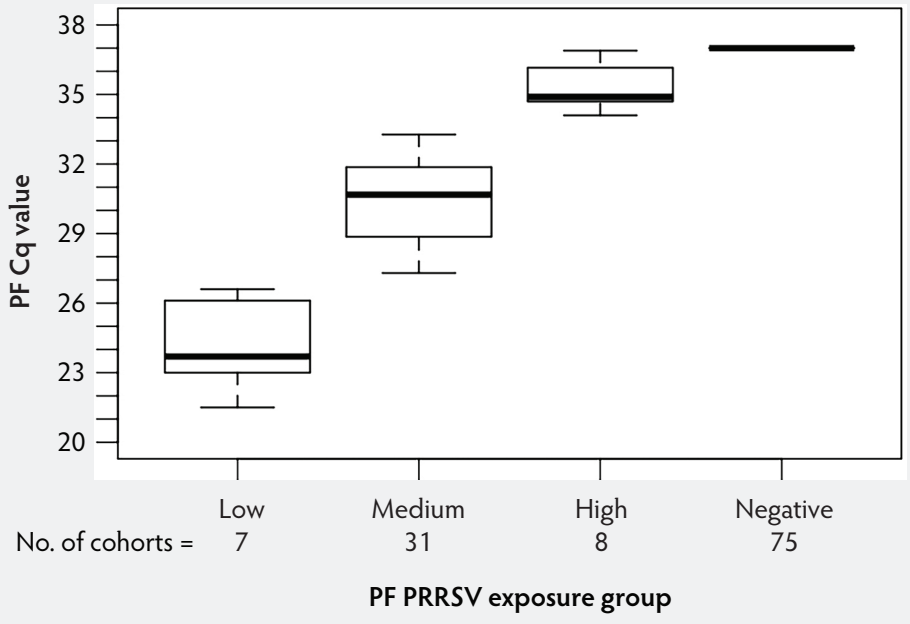
Based on PF testing in the breeding herds, the number of cohorts and Cq value distribution for each PRRSV exposure group is presented in Figure 2. The mortality distribution for 121 cohorts according to PF PRRSV exposure group is presented in Figure 3. The number of piglets that contributed to a PF sample per farm and week ranged from 400 to 2300 piglets. The lowest Cq value obtained from PF samples was 21.5 (Figure 2).

Pig cohorts belonging to the low exposure group had significantly higher nursery mortality than other groups ($P < .001$; Table 1). Mortality differences between the medium, high, and negative groups were small as compared to differences between these groups and the low group. The overall mean mortality for the low group was 3.40 percentage points higher than all other groups. There was no significant difference in the mean mortality for high vs negative exposure groups.

Based on OF testing in the nursery, the number of cohorts and Cq value distribution for each PRRSV shedding group is presented in Figure 4. The mortality distribution for each OF PRRSV shedding group is presented in Figure 5. Nursery mortality comparisons between PRRSV shedding groups for 41 cohorts is shown in Table 2. There was no nursery cohort classified in the low shedding group (Figure 4). Nursery cohorts in the medium shedding group had 1.33 percentage points higher mortality than those in the high group ($P < .001$) and 1.57 percentage points higher mortality than those in the negative group ($P < .001$). Nursery cohorts in the high shedding group had 0.25% numerically higher mortality compared to negative cohorts ($P = .18$).

The relationship between qRT-PCR results from PF and OF samples and nursery mortality is presented in Figure 6. Cohorts categorized as low PRRSV exposure and medium PRRSV shedding had the highest

Figure 2: Distribution of cohorts and PF Cq values for each PRRSV exposure group using qRT-PCR. PF = processing fluids; Cq = quantification cycle; PRRSV = porcine reproductive and respiratory syndrome virus; qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction.



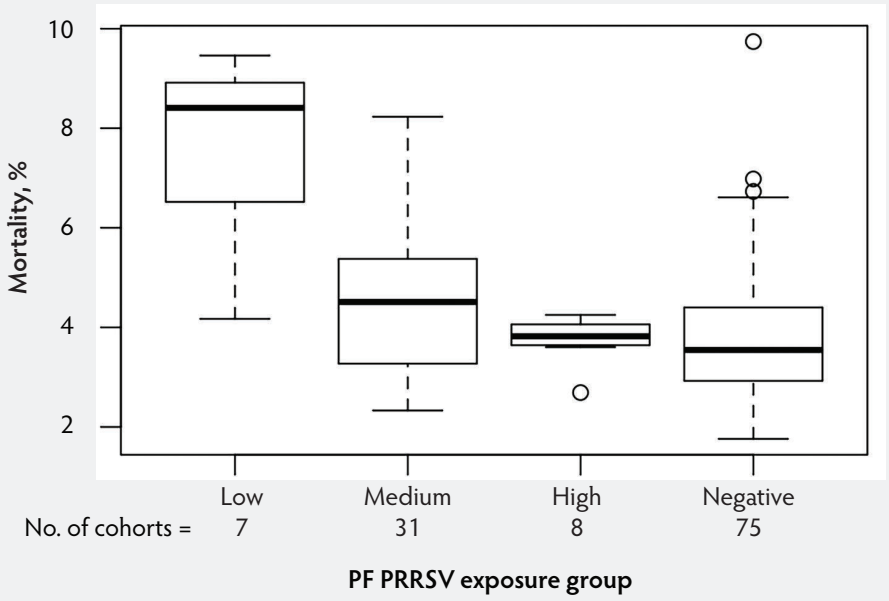
mortality compared to all other groups. Cohorts with medium exposure and medium shedding had higher mortality compared to cohorts with medium exposure and negative shedding. Cohorts with negative exposure had similar mean mortality in all 3 nursery shedding groups, but cohorts that had negative exposure and negative shedding had the smallest variability in mortality. There was only one cohort that had high exposure and medium shedding.

The overall crude agreement of PRRSV by qRT-PCR results between PF and OF was 63.41%. The Kappa agreement test, which excludes the agreement by chance, was 0.2398 as shown in Figure 7.

A total of 66 sets of OF were tested for PRRSV by qRT-PCR individually and in pools of 4:1. The specificity obtained for this analysis was 100% and the sensitivity was 76.92% (Figure 8). The positive predictive value was 100% and negative predictive value was 94.64%. There was a failure to detect PRRSV RNA in 3 pooled OF samples where at least one of the individual samples contributing to the pool returned a positive on individual testing. The qRT-PCR Cq value for the individual positive samples that contributed to the PCR-negative pools ranged from 34.67 to 36.75. In the same cohorts, a negative result on PF samples was previously obtained.

Ten ORF-5 PRRSV sequences were performed in 6 PF samples collected from 6 different sow farms and 4 OF samples from 2 different commingling flows representing piglets from 4 different sow farms. Samples with low and medium Cq values were used for sequencing. Four of six PF samples and all OF samples returned a restriction fragment length polymorphism (RFLP) vaccine-like type 2-5-2 sequence with more than 98% similarity with the PRRS Ingelvac modified-live virus vaccine strain. One PF sample returned an RFLP wild-type 1-1-1 sequence and one sample (Cq = 32.21) failed to be sequenced.

Figure 3: Distribution of nursery mortality rate for each PF PRRSV exposure group. PF = processing fluids; PRRSV = porcine reproductive and respiratory syndrome virus.



Discussion

This was a prospective study using PF PRRSV qRT-PCR Cq values to classify 121 cohorts according to PRRSV exposure status in the breeding herd. This status was used as an indicator for subsequent nursery mortality. Exposure groups classified as low had higher mortality than all other exposure groups. In this study the cumulative nursery mortality was 3.99 percentage points higher for the low compared to the high PRRSV exposure group and 3.76 percentage points higher when comparing the low with the negative PRRSV exposure groups. Additionally, 41 cohorts were tested for PRRSV RNA by qRT-PCR at 6 weeks of age using OF samples to assess the level of PRRSV shedding in the nursery. Associations between PRRSV exposure in the breeding herd and PRRSV shedding in the nursery, as well as nursery mortality, were investigated. To the best of our knowledge, this was the first work describing the agreement between PF and subsequent nursery OF results for PRRSV qRT-PCR testing obtained from the same cohorts. Using qRT-PCR for PRRSV detection, PF results had a fair agreement with OF results ($\kappa = 0.2398$)

Table 1: Pairwise comparisons of nursery mortality between PRRSV exposure groups determined by PF testing

PRRSV exposure group comparison	Mean difference in mortality (95% confidence limits)	Tukey *P* value
Low vs Medium	3.28 (2.88, 3.46)	< .001
Low vs High	3.99 (3.52, 4.12)	< .001
Low vs Negative	3.76 (3.48, 4.03)	< .001
Medium vs High	0.70 (0.46, 0.83)	< .001
Medium vs Negative	0.47 (0.45, 0.73)	< .001
High vs Negative	-0.23 (0.21, 0.01)	.13

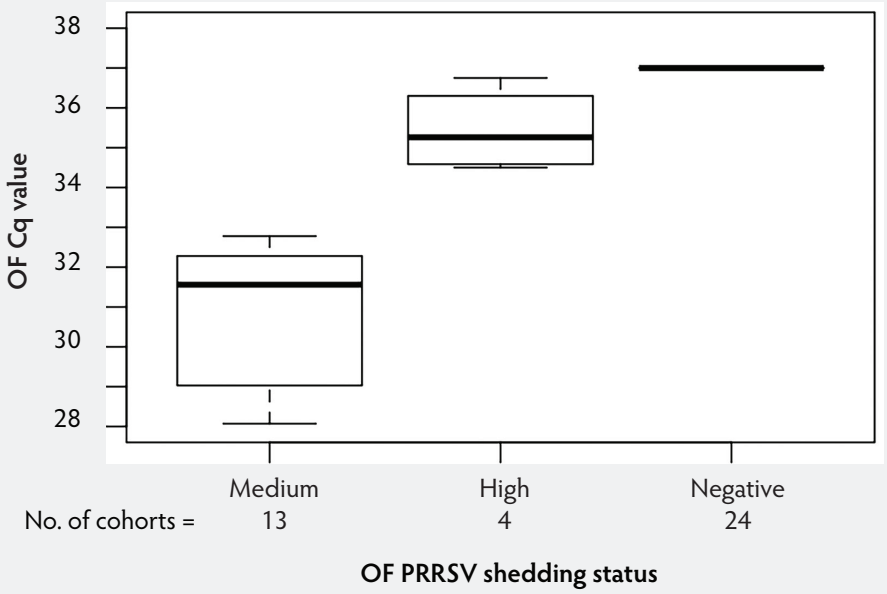
PRRSV = porcine reproductive and respiratory syndrome virus; PF = processing fluids.

Table 2: Pairwise comparisons of nursery mortality between PRRSV shedding groups determined by OF testing

PRRSV shedding group comparison	Mean difference in mortality (95% confidence limits)	Tukey *P* value
Medium vs High	1.33 (1.03, 1.62)	< .001
Medium vs Negative	1.57 (1.38, 1.77)	< .001
High vs Negative	0.25 (0.02, 0.52)	.18

PRRSV = porcine reproductive and respiratory syndrome virus; OF = oral fluids.

Figure 4: Distribution of cohorts and OR Cq values for each PRRSV shedding group using qRT-PCR. OF = oral fluids; Cq = quantification cycle; PRRSV = porcine reproductive and respiratory syndrome virus; qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction.



and are most likely to be influenced by the interval between collections, differences in the sample matrices, or both. Cohort classification of shedding status in the nursery tended to differ from the sow herd exposure status classification. All cohorts classified as low exposure groups using PF samples were classified as medium shedding status using OF samples. Also, this study evaluated the impacts of 4:1 OF sample pooling tested for PRRSV RNA by qRT-PCR. Pooling OF samples in a 4:1 ratio proved to be a practical approach for monitoring PRRSV in endemic herds. When the pooled sample failed to detect the positive result of an individual sample, the Cq value on the individual sample was > 34, and the high exposure group did not differ in cumulative nursery mortality from the negative exposure group.

The overall findings indicate that PRRSV qRT-PCR Cq values from PF samples can be used as an indicator for expected cumulative nursery mortality differences. As the Cq value of PF samples decreased, the subsequent overall nursery mortality increased. The most significant difference was the low PRRSV exposure group, which had the highest mortality among all exposure groups. As presented in Table 1, the higher mortality of the low exposure group when compared with all other exposure groups indicates that Cq values can be used as an inversely proportional predictor of nursery mortality, ie, the lower the Cq value the higher the expected nursery mortality. The polymerase chain reaction assay measures the amount of nucleic acid detected in the samples, but does not indicate the presence of infectious material.[21] Results from qRT-PCR assays were used in studies to determine virulence of PRRSV strains[22] and efficacy of vaccines.[23] In general, the lower the Cq value, the higher the expected concentration of a pathogen's genomic copies. For the low PRRSV exposure group, the expected higher pathogen concentration in the samples was associated with the increased subsequent nursery mortality. The high PRRSV exposure group, represented by the last 10-fold increase in the detection of PRRSV by qRT-PCR, had similar mean mortality as the negative exposure group, suggesting that a Cq value above 34 is indicative of a lower concentration of PRRSV genomic copies in the PF sample and, therefore, lower virus circulation among the newborn population with a small effect on nursery mortality. Alternatively, it may only be detection of PRRSV genetic material without the presence of infectious virus. Similarly, when considering the level of PRRSV shedding in the nursery, which was measured using qRT-PCR on OF samples, cohorts that had the smallest Cq values (medium Cq shedding group) had higher mortality rates than high or negative shedding groups.

Considering a mortality difference of 3.76 between the low and negative PRRSV exposure groups and that $40.89 was the average estimated purchase price[24] for a 12 lb piglet between January to July 2018, this increased mortality represents a loss of $153.75 (3.76 × $40.89) per 100 head placed in the nursery. The qRT-PCR Cq values of PF samples can be used as an indicative tool to develop strategic PRRSV vaccination interventions[25-27] and management practices for different exposure groups to reduce significant economic production losses.

Co-infections between PRRSV and other pathogens are commonly reported. The most frequently reported co-infection agents include influenza A virus, *Streptococcus suis*,

porcine circoviruses, *Haemophilus parasuis, Mycoplasma hyopneumoniae,* and *Pasteurella multocida.*[28,29] Thus, watching for infection with other pathogens and proper treatment may help to prevent mortality. Additionally, measures that can be used to reduce the PRRSV spread and circulation in contaminated cohorts and reduce mortality include changing needles between animals when administering treatment,[30] using needle-free technologies,[31] adoption of all-in/all-out nursery flows with proper facility sanitation and disinfection between cohorts,[32,33] and adoption of management changes to reduce exposure to bacteria to eliminate losses in the farrowing house.[34]

Overall, the Cq value results from PF samples were lower than OF samples, indicating that the concentration of PRRSV RNA present in each sample type is different. All the cohorts (n = 7) that were classified as a low PRRSV exposure group in the breeding herd moved to a medium nursery shedding group based on OF testing. There was a fair agreement of binary qRT-PCR results obtained between PF and subsequent OF samples. This fair agreement could be explained by the time difference in the collection, as samples were collected with a five-week interval. Other potential interference was the sample size, whereas PF collection from all castrated litters during the collection days potentially included more piglets than collection of 4 OF samples. Additionally, it is biologically possible that positive piglets tested using PF were not positive at the moment of OF collection since most individual piglets are likely no longer viremic after 28 days post PRRSV infection.[31,35,36] Another possibility is that the number of nursery pens sampled (n = 4) was not sufficient to detect a positive sample when prevalence in the barn is low. In this study, it is plausible that a positive or negative result on PF was not a good indicator for the subsequent OF result. Nevertheless, this was a field-based study, and thus it is possible to have inhibitors present in OF samples which are not present in PF samples, and vice versa, influencing the polymerase chain reaction outcome. However, qRT-PCR Cq values obtained from PF samples were used to categorize cohorts according to PRRSV exposure and were successfully used as an explanatory indicator for cumulative nursery mortality.

Figure 5: Distribution of nursery mortality rate for each OF PRRSV shedding group. OF = oral fluids; PRRSV = porcine reproductive and respiratory syndrome virus.

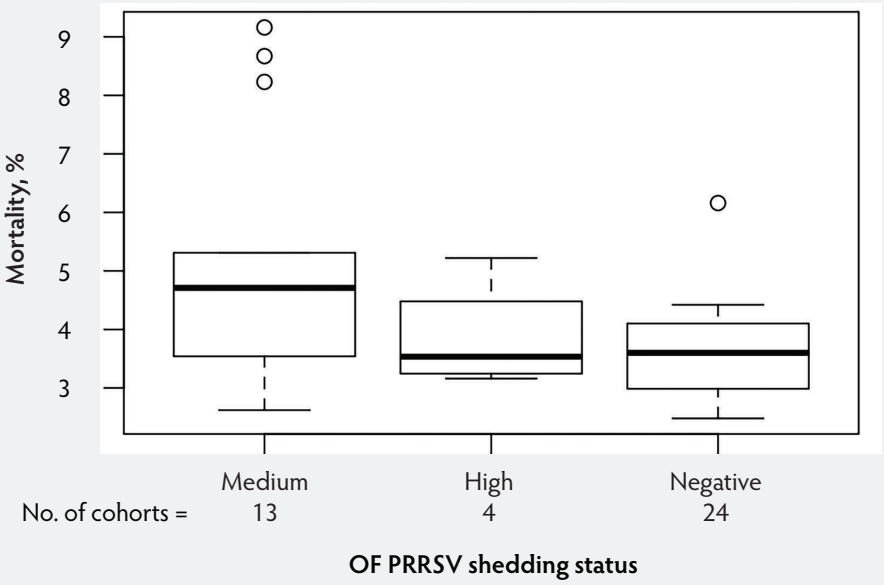
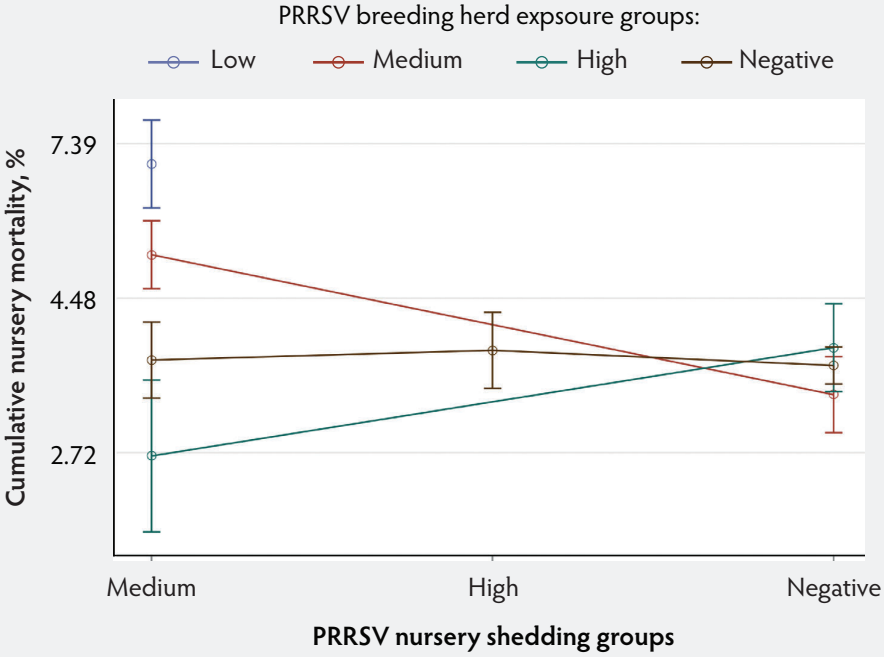


Figure 6: Least squares means of nursery mortality by PRRSV exposure in the breeding herd (based on PF testing results; colored lines) and subsequent PRRSV shedding in the nursery (based on OF testing results; on x-axis). Circles represent the mean group mortality and the whiskers represent 2 SD of the mean. PRRSV = porcine reproductive and respiratory syndrome virus; PF = processing fluids; OF = oral fluids.



The pooling effect on the probability of PRRSV RNA detection by qRT-PCR has been investigated in other studies for serum and blood swab,[37] semen,[38] and OF.[39,40] The described pooling of individual samples in a 5:1 ratio comes with the expense of losing sensitivity to detect PRRSV, but allows to cover more individual samples in a qRT-PCR test. Pooling of PF was also described,[41] and the pooling of PF samples from the room of collection did not reduce the sensitivity to detect a PRRSV-positive sample when compared to a pooled PF sample from an individual litter. For the current study, pooling OF in a factor of 4:1 resulted in a specificity of 100% and a sensitivity of 76.92%. For all 3 cases where individual results did not agree with the pooled sample results, the Cq value of the individual sample was above 34, but the cohort was classified in the high nursery shedding group. This finding aligned with previous work where pooled OF samples having Cq > 34 had a diagnostic sensitivity of only 27%.[40] Cohorts classified as high for nursery shedding did not differ from negative cohorts in percent cumulative mortality. Pooling OF samples for PRRSV shedding monitoring purposes could be a good approach to allow inclusion of a larger number of piglets for PRRSV testing. When a positive result was obtained from the pooled sample, it represented a truly positive aggregated sample according to the 100% positive predictive value. For the 3 samples which did not agree on the individual versus pooled testing, two factors may have contributed. First, the PRRSV prevalence within the cohort had been low resulting in the failure to detect the virus. Previous work did not find a difference for detecting PRRSV RNA using OF in pools of 3:1 or 6:1.[42] Second, the OF pooling effect could have potentially diluted the positive sample increasing the final Cq value above the negative cut-off limit of 37 and, as a consequence, classifying the sample as negative. The effect of OF sample pooling on the increase of Cq value was not investigated.

The use of PF as a sample type to characterize weekly batches of suckling piglets according to PRRSV exposure status in breeding herds was demonstrated as a practical and efficient approach, serving as a good indicator for subsequent cumulative mortality in the nursery. Being aware of this relationship aids the development of strategies for disease prevention and to minimize losses caused by PRRSV.

Implications

Under the conditions of this study:

- Processing fluid can be used to characterize PRRSV exposure of newborn pigs.
- Low PRRSV exposure groups had higher nursery mortality than all other groups.
- Pools of 4:1 OF samples were useful to monitor PRRSV status.

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Conflict of interest

Dr Jose Angulo is a member of the Zoetis US Pork Technical Services team. Drs Rebecca Robbins and Luc Dufresne are members of Seaboard Foods, LLC. Zoetis and Seaboard Foods, LLC provided diagnostic funding for this project.

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Figure 7: Crude agreement and Kappa analysis for PRRSV qRT-PCR results obtained from processing fluid and oral fluid from the same cohort. Crude agreement varies from 0 to 100. Kappa varies from 0 to 1. For crude agreement and Kappa agreement zero means no agreement and 1 means perfect agreement. PRRSV = porcine reproductive and respiratory syndrome virus; qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction.

		Oral fluid result: +	Oral fluid result: -	
Processing fluid result	+	9	7	16
	-	8	17	25
		17	24	41

Crude agreement	0.6341
Kappa agreement	0.2398

Figure 8: Sensitivity and specificity analysis for oral fluid samples tested for PRRSV by qRT-PCR individually and in pools of 4:1. PRRSV = porcine reproductive and respiratory syndrome virus; qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction.

		Individual samples: +	Individual samples: -	
Pooled samples	+	10	0	10
	-	3	53	56
		13	53	66

Sensitivity	76.92%
Specificity	100%

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Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°F	°C
32	0
50	10
60	15.5
61	16
65	18.3
70	21.1
75	23.8
80	26.6
82	28
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100

°F = (°C × 9/5) + 32
°C = (°F - 32) × 5/9

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	135
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg
1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne
1 ppm = 1 mg/L