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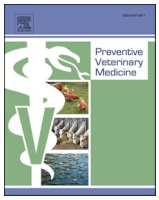

Infection dynamics and incidence of wild-type porcine reproductive and respiratory syndrome virus in growing pig herds in the U.S. Midwest

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) infections greatly impact the health and productivity of growing pigs. The introduction and persistence of wild-type PRRSV (WT-PRRSV) strains in growing pig populations is poorly understood. In an observational prospective cohort study, we monitored and surveyed 63 wean-to-finish (WTF) herds across 10 companies located in medium to high pig dense areas in the U.S. Midwest. All herds received weaned pigs from PRRSV-negative or positive-stable breeding herds. Herds were monitored monthly using oral fluids collected following a fixed spatial sampling regime and samples were tested by PRRSV ELISA, RT-PCR and ORF5 sequencing. In most (90%) of the herds, pigs were vaccinated with PRRSV modified-live vaccines either at processing, weaning or shortly after weaning. Wild type PRRSV (WT-PRRSV) infections were defined by the criterion of having more than 2% nucleotide differences in the ORF-5 region compared with reference vaccine strain sequences. Wild type PRRSV was detected in 42% of the herds with infections being more prevalent in the mid to late growing period, with a mean of 20 weeks post placement. Nineteen distinct WT-PRRSV were identified in seven out of 10 production companies with an average of 3 distinct WT-PRRSV strains per company. Vaccinated WTF herds with and without WT-PRRSV detection were compared to each other showing different PCR and ELISA infection patterns. Close-out mortality in vaccinated herds with WT-PRRSV was numerically higher (6.5%) than mortality in those sites where WT-PRRSV was not detected (5.0%) ($p = 0.07$). Mortality was also higher (10.5%) when WT-PRRSV was detected earlier at eight weeks post-placement compared to late finishing at 20 and 25 weeks post-placement, 2.9% and 4.5% respectively ($p = 0.017$). Overall, this study sheds light on WT-PRRSV infection dynamics in vaccinated populations of growing pigs, reinforces the importance of biosecurity practices in this phase of production and calls for better understanding of risk factors associated with PRRSV introductions in growing pig sites.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is arguably the most important swine disease in the United States due to its detrimental effect on pig health and productivity, and consequently its enormous economic impact on the swine industry (Holtkamp et al., 2013). PRRS virus (PRRSV) causes severe clinical disease in all phases of swine production including breeding and growing pigs. In growing pigs, PRRSV causes inappetence, lethargy, and dyspnea, resulting in lower average daily gain and higher feed efficiency. The economic impact of PRRS in the United States was estimated to be $664 million annually, with 54% of the losses attributed to infections of growing pigs (Holtkamp et al., 2013).

There are knowledge gaps in PRRSV epidemiology in growing pigs, the understanding of risk factors for PRRSV introduction into herds, and the importance of PRRSV transmission from growing pig sites to sow farms (Galvis et al., 2022). Growing pig populations can be reservoirs and amplifiers of PRRSV that may represent a significant threat to sow farms' PRRSV stability (Jiang et al., 2021). Factors such as farm proximity, geographic terrain, pig movement, pig density and poor external biosecurity are factors associated with the spread of PRRSV between farms (Jara et al., 2021; VanderWaal et al., 2020; Arruda et al., 2017; Dee et al., 2003, 2004). PRRSV outbreak investigations in growing pigs are particularly difficult to decipher since there are often complex temporo-spatial relationships and incomplete understanding of all direct and indirect connectivity factors between sites. Furthermore, although

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airborne transmission has been documented in some circumstances (Torremorell et al., 1997; Dee et al., 2009), its importance in PRRSV infections in growing pigs is not well understood (Arruda, . et al., 2019). One important epidemiological feature of PRRSV is the prolonged duration of infection of individual pigs, which enhances likelihood of endemic infection at the population level. PRRSV can be detected for 165 days post infection in tonsils and lymphoid tissues (Fangman et al., 2007). Comingling of carrier and susceptible pigs through the introduction of replacement animals, birth, or mixing of sources of different PRRSV status (Wills et al., 2003; Hess et al., 2019), and other management factors such as low ventilation rates (Fablet et al., 2016), facilitate the perpetuation of PRRSV infections in herds.

Pig vaccination with PRRSV modified-live virus (MLV) vaccines is common in North America (Galvis et al., 2022) and MLV vaccines are considered beneficial to control the disease and to mitigate its economic impact (Renukaradhya et al., 2015). However, vaccination with MLV vaccines adds complexity to surveillance programs due to the need to differentiate vaccine viruses from wild type PRRSV strains, which makes it difficult to understand PRRSV infection dynamics and introduction of new strains into the herds.

In this study, we prospectively analyzed PRRSV infection dynamics in growing pigs in wean to finish sites from the Midwest region in the United States. We describe infection dynamics of both, wild type PRRSV (WT-PRRSV) and MLV vaccine strains and focus our main analysis on timing of WT-PRRSV introduction and impact on mortality.

2. Material and methods

2.1. Study design

Sixty-three wean-to-finish (WTF) herds from 10 swine production companies located in the US Midwest were enrolled in a prospective cohort study from September 2017 to December 2018. The herds were recruited throughout the year and the number of herds was selected by convenience primarily considering logistics for implementation, willingness to participate, and budget limitations. The herd inclusion criteria included: 1) pigs were sourced from PRRSV negative or stable breeding herds as specified by the guidelines of the American Association of Swine Veterinarians (AASV) PRRSV breeding herd classification (Holtkamp et al., 2011); 2) pigs were placed in all-in/all-out WTF sites located in geographical areas considered of medium to high pig density (10–20) pig sites within 4.8 kilometers; 3) producer willingness to collect monthly oral fluid samples for PRRSV testing; and 4) producer willingness to share site-level information including herd demographics, site characteristics, and production records for the specific pig groups in the study. To select the herds, we contacted veterinarians in production companies in the Midwest (Iowa and Minnesota) and asked them to provide a list of herds that would fit the herd selection criteria, including timelines for filling the sites and geographical coordinates for each site. The number of pig sites within 1, 3 and 5 miles (1.6, 4.8 and 8.0 kilometers) of each study site was determined from public accessible feedlot databases of the Minnesota Pollution Control Agency (MPCA, https://gisdata.mn.gov) and the Iowa Department of Natural Resources (IDNR, https://www.iowadnr.gov Animal feeding operations).

Once herds were selected, an enrollment questionnaire with 14 questions was filled out by the herd veterinarian using Google forms to obtain site level information. Another questionnaire with 24 additional questions was administered at approximately 2 weeks post placement of the pigs at the WTF sites. All herds in the study received an individual code and data were coded confidentially. Data from questionnaires captured information about site characteristics and management practices such as location, sow farm source and PRRS status, vaccination protocols, downtime period before filling the sites, number of days to fill the site, first fill date, number of pig sources, pig flow, number of pigs housed, barns and pens per site. Sixty-one WTF sites also provided closeout data of mortality in each barn at the time of closing the group.

2.2. Sample collection and testing

Sample collection procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC) according to protocol number 1510–33054 A. Researchers provided written instructions on how to select the pig pens within the site for sampling, how to collect the samples, and how to submit the samples after collection.

Eight oral fluid samples were collected from each group at each WTF site approximately every 4 weeks. Written instructions were provided so that the same pens of pigs were sampled each time following fixed spatial sampling recommendations (Rotolo et al., 2017). Briefly, pens were distributed throughout the barn(s) to obtain a representative sample of the pigs on-site, then pens were selected in each barn, i.e., for a site with two barns, 4 pens were selected in each barn and a rope was placed in each of the pens of each barn. Location of the pens was determined by identifying pens distributed equally over the length of the barn and adding an identification mark in each pen for further samplings during the study. Oral fluids were collected using cotton ropes as previously described (Prickett et al., 2008). Briefly, ropes were placed hanging in the pens for 30 min for the pigs to chew and deposit their oral secretions on them. Oral fluids were extracted from the ropes manually and after collection, they were refrigerated using ice packs and shipped to the University of Minnesota. On arrival, samples were processed, aliquoted and stored frozen at − 80 C until testing.

Samples were tested individually using a PRRSV RT-PCR as previously described (Nirmala et al., 2021). Samples with cycle threshold (Ct) of $\leq$ 37 were considered positive, while samples with Ct $>$ 37 were considered negative. The sample with the lowest Ct (if $<$ 33) at each sampling event was further characterized by sequencing the PRRSV open reading frame 5 (ORF-5) directly from the oral fluid sample. Sanger sequencing was performed following the procedures at the University of Minnesota Veterinary Diagnostic Laboratory (Alonso et al., 2013) and sequences analyzed. A predictive restriction fragment length polymorphism (RFLP) was also obtained (Wesley et al., 1998). Sequences were aligned using MegAlign from DNASTAR software (Version 15.1.0) using Clustal W method and compared with PRRSV reference strains from the commercial modified live vaccines present in the market at the time of the study. ORF-5 vaccine reference strain sequences were obtained from Genbank: a) Ingelvac PRRS MLV accession number AF066183, b) Ingelvac PRRS ATP accession number DQ988080 and c) Fostera PRRS accession number JB398244. Sequences were considered to be WT-PRRSV if they had equal or more than 2% of nucleotide differences from any of the MLV vaccines used in the study. To validate that a 2% nucleotide difference between ORF5 sequences obtained from vaccinated pigs in our study would differentiate vaccine from WT-PRRS strains, an individual plot value distribution of the genetic distance between ORF5 sequences and each reference vaccine sequence was created. Sequences classified as MLV clustered together and had $\leq$ 2% nucleotide differences from the respective reference MLV sequences (Fig. 3). Sequences $>$ 2% nucleotide differences were considered WT-PRRSV.

Oral fluids were further tested for antibodies using the PRRS IDEXX ELISA test (HerdCheck, IDEXX Laboratories Inc, Westbrook, ME). Testing procedures followed manufacturer instructions. Briefly, samples were diluted 1:2 with sample diluent. 100 μL of each diluted sample were transferred to antigen-coated plates, with undiluted positive and negative controls, and incubated for 2 h at 25 °C. Plates were washed 5 times with 350 μL wash solution per well using BioTek 405 TSU plate washer (BioTek, Winooski, VT, USA). 100 μL of conjugate solution was added per well, then incubated for 30 min at 25 °C, and washed 5 times. 100 μL of TMB Substrate N.12 was added per well, incubated for 15 min at 25 °C, and then color development was stopped with 100 μL stop solution N.3 per well. The absorbance (450–650 nm) was measured and recorded using BioTek Synergy H1, (BioTek, Winooski, VT, USA). Values with sample to positive (S/P) ratios $>$ 1.0 were considered positive

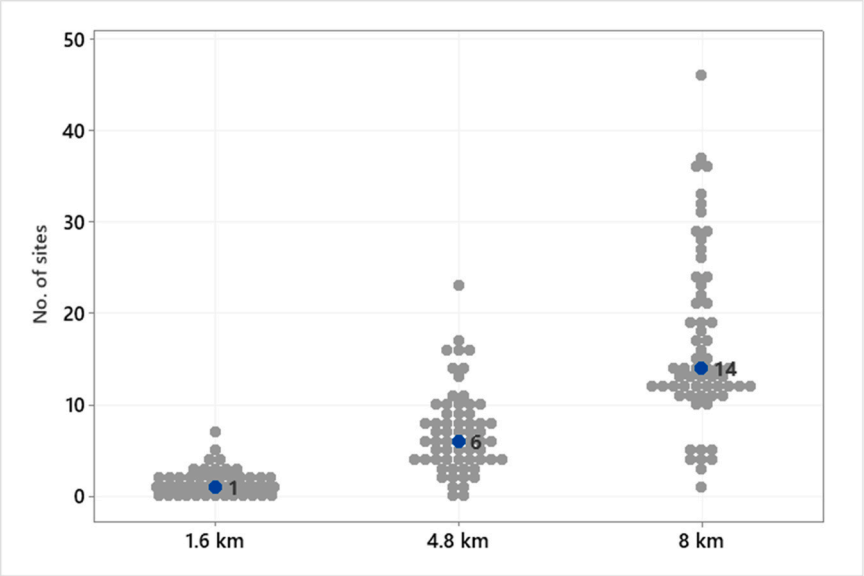

Fig. 1. Number of pig sites within 1.6, 4.8 and 8 kilometers (1, 3 and 5 miles) of the wean to finish herds enrolled in the study. Numbers within figure indicate the median of pig sites within each of the distances.

while values < 1.0 were considered negative (Henao-Diaz et al., 2021).

2.3. Data analysis

Data were organized in spreadsheets (Microsoft) to obtain data summaries and descriptive statistics using the complete dataset (Minitab 20.1.1. State College, PA, USA). To evaluate incidence of WT-PRRSV infection, three WTF herds that tested positive at the first sampling event were excluded from the analysis since the results suggested that the pigs were positive on arrival, thus did not meet the inclusion criteria. The remaining 60 WTF herds were included in the statistical analysis. A descriptive analysis of all RT-PCR results by sampling event from each herd was visualized using individual plot values. The proportion of ORF5-PRRSV sequences classified as either WT-PRRSV or vaccine-like in each sampling event was compared using analysis of Chi-square. ELISA (S/P ratios) and PRRSV PCR (Ct) results from oral fluid samples were analyzed from herds with and without WT-PRRSV detection together to understand infection dynamics. Negative Ct values were assigned a Ct value of 45 for calculation purposes. Kruskal-Wallis pairwise comparisons using Bonferroni corrections were used to compare incidence levels of WT-PRRSV from vaccinated sites at each sampling point. In addition, a Kaplan-Meier survival analysis was done to determine time to first WT-PRRSV detection.

The genetic distance between sequences classified as MLV and wild-type was compared using Man-Whitney test, then Wilcoxon signed rank test was used to determine whether the percentage nucleotide difference obtained from strains classified as MLV had less than 2% heterology.

The impact of WT-PRRSV detection on mortality was analyzed using data from 58 close-outs, comparing reported mortality from sites with and without WT-PRRSV detection, using permutations based on a randomization test for 2-sample means (Minitab 20.1.1, College PA, USA) where WTF site was the individual measure with binary explanatory variables (WT-PRRSV detected, WT-PRRSV not-detected) and the response variable was mortality data obtained from closeouts. In addition, closeout mortality from groups when WT-PRRSV was detected for the first-time post-placement was analyzed through Kruskal-Wallis pairwise comparisons using Bonferroni corrections.

Table 1
Summary description of the sixty-three wean to finish herds enrolled in the study.

Site information	Median	Min-Max
Number of pig spaces per site	4560	1200–9600
Number of barns per site	2	1–6
Number of pig spaces per barn	2400	700–5000
PRRS Vaccination	57 (90%)	Vaccinated
	6 (10%)	Not vaccinated
	No. of sites per production company (%)	**Production companies**
Production companies (n = 10)	16 (25%)	A
	3 (5%)	B
	1 (2%)	C
	12 (19%)	D
	14 (22%)	E
	5 (8%)	F
	4 (6%)	G
	3 (5%)	H
	1 (2%)	I
	4 (6%)	J

3. Results

3.1. Herd characteristics

The 63 WTF herds were located in the states of Iowa and Minnesota, which are the top pig producing states in the U.S. (Census of Agriculture, 2017). Study sites had median of 1 (min 0, max 7), 6 (min 0, max 23) and 14 (min 1, max 45) neighboring pig farms within distances of 1.6, 4.8, and 8 kilometers (1, 3 and 5 miles), respectively (Fig. 1). Table 1 summarizes the herd characteristics and PRRSV vaccination status. Individual production companies contributed from 1 to 16 WTF herds. Thirty-two (51%) breeding herds that sourced the pigs into the WTF herds were PRRSV positive stable, negative, naïve, or did not vaccinate sows, while 31 (49%) of the breeding herds vaccinated sows. In addition, 49 sites (78%) received pigs from a single breeding herd, while 14 (22%) received pigs from two or more. Out of the 57 (90%) WTF herds that had vaccinated pigs, 24 (42%) of the herds had pigs that were either vaccinated prior to weaning at processing or around weaning age. Thirty-three (58%) herds vaccinated pigs at placement into the WTF or

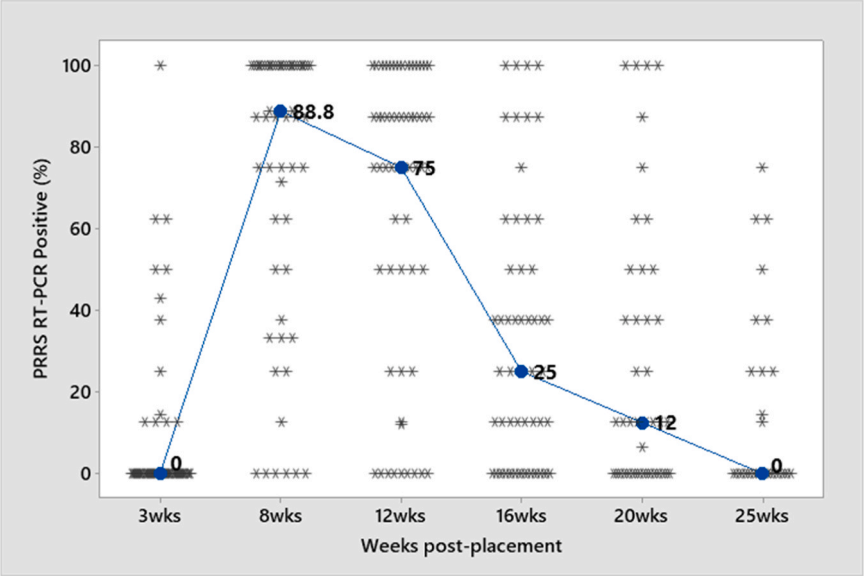

Fig. 2. Distribution of the prevalence of positive porcine reproductive and respiratory syndrome (PRRS) virus RT-PCR results for each wean to finish herd (n = 60) at each sampling event. Numbers in bold are the median prevalence at each sampling event (blue line).

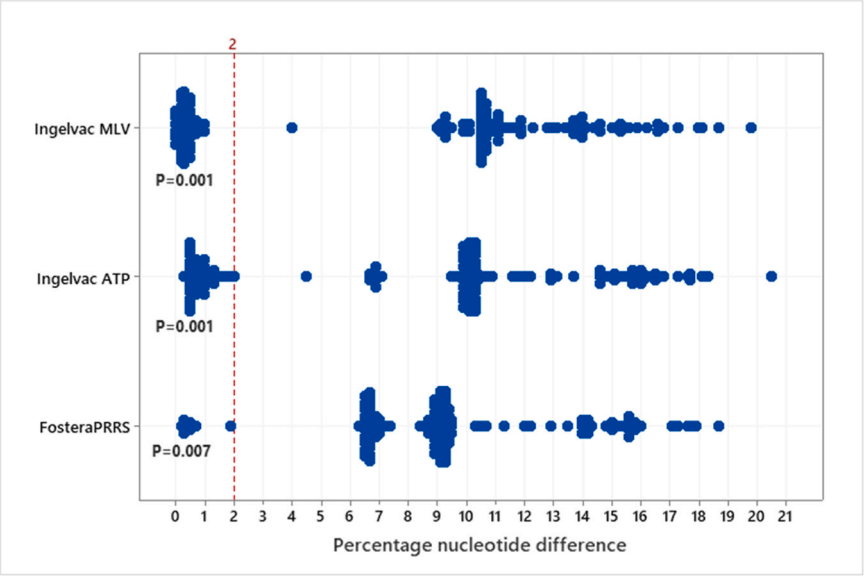

Fig. 3. Individual values of percentages of genetic distances of porcine reproductive and respiratory syndrome virus modified live vaccines (PRRSV MLVs) and wild type PRRSV (WT-PRRSV). A 2% ORF-5 genetic difference was selected to differentiate PRRSV MLVs from WT-PRRSV based on the Wilcoxon signed rank test.

within 1–3 weeks post placement. All vaccinated pigs were vaccinated with one of the commercial PRRSV vaccines available at the time of this study which included Ingelvac PRRS MLV and Ingelvac PRRS ATP (Boehringer Ingelheim Vetmedica, St. Joseph MO), and Fostera PRRS MLV (Zoetis, Parsippany NJ).

3.2. PRRSV active surveillance results

All WTF herds completed sampling until pigs were moved out to market with the exception of two herds that stopped sampling after the first detection of WT-PRRSV at 3rd and 4th sampling event. A total of 2584 oral fluid samples were collected between September 2017 and November 2018. The median number of weeks post-placement at each sampling event was 3, 8, 12, 16, 20 and 25. Sixty WTF herds fulfilled the study inclusion criteria for being sourced from PRRSV negative or stable breeding herds while 3 herds were confirmed WT-PRRSV positive at placement and were excluded from analyses. WT-PRRSV was detected in 25 (42%) of the herds. Herds that tested positive for the modified live vaccine at 3 weeks remained in the study. Only PRRSV type-2 was confirmed in the herds.

Fig. 2 illustrates the number of positive samples (vaccine and WT-PRRSV) at each sampling event in each WTF herd. The highest positivity rate by PRRSV RT-PCR was observed in the second sampling event at 8 weeks post-placement approximately.

3.3. ORF5 PRRSV sequencing results

Thirty-six out of 139 (26%) sequences were classified as WT-PRRSV (avg Ct 28.8) and grouped in 19 distinct WT-PRRSV phylogenetic clusters (Table 2). There was an average of 3 distinct WT-PRRSV clusters/variants per production company. Grouping of the sequences in viral groups is shown in the phylogenic tree in Fig. 5 with vaccine sequences

Table 2
Number of open reading frame 5 (ORF-5) sequences classified as wild-type porcine reproductive and respiratory syndrome (WT-PRRSV) or vaccine-like viruses, percentage of nucleotide differences and minimum and maximum range between sequences, by production company.

Production company (Total no. sequences)	No. of herds with sequences* ** *	No. WT-PRRSV sequences	No. distinct WT-PRRSV viruses*	Average % difference between WT-PRRSV strains (min-max)	No. vaccine-like sequences	Average % difference between vaccine-like sequences Avg. (min-max)
A (n = 33)	14/16	12	6	8.00 (0–17.9)	21	0.64 (0–1.8)
B (n = 6)	3/3	2	1	0.2 (n/a)	4	0.47 (0.2–0.8)
C (n = 5)	1/1	1	1	n/a (n/a)	4	0.72 (0–1.2)
D (n = 21)	10/12	8	4	10.14 (0.5–20.3)	13	0.29 (0–0.9)
E (n = 40)	14/14	9	5	8.4 (0.2–14.4)	31	0.4 (0–1.5)
F (n = 13)	4/5	0	0	n/a (n/a)	13	0.9 (0.2.4)
G (n = 13)	4/4	2	1	0 (n/a)	11	0.69 (0–1.3)
H (n = 6)	3/3	0	0	n/a	6	4.88 (0.2–9.6)* **
I (n = 0)	0/1	0	0	n/a	0	n/a
J (n = 2 **)	2/4	2	1	n/a	0	n/a
Total (n = 139)	42/63	36 (26%)	19	5.34	103 (74%)	1.12

n/a: not applicable due to number of sequences = 0 or 1
*Sequences were considered genetically distinct when percentage nucleotide homology between sequences was > 2%.
* *One sequence is missing in the dendrogram but the production company reported it as wild-type PRRSV.
* ** Two distinct vaccines were used in production company H.
* ** * No. of herds/total number of herds

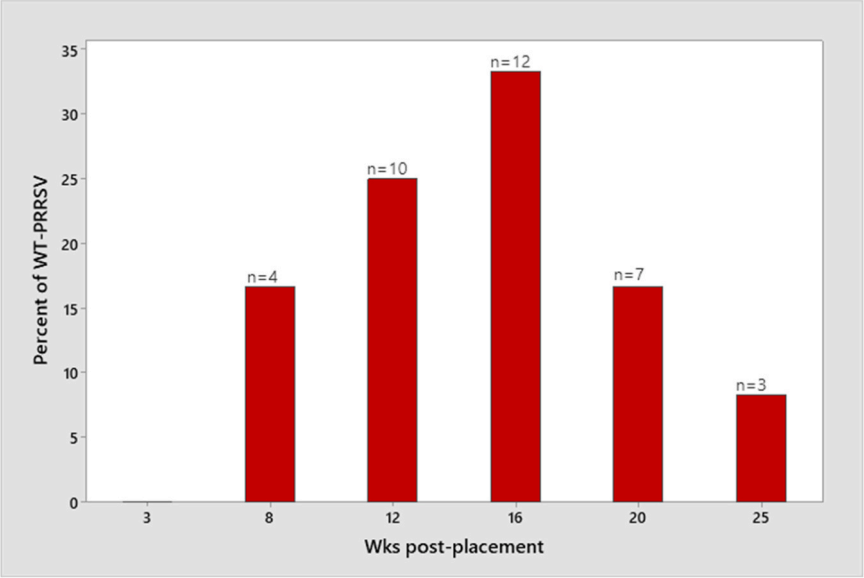

Fig. 4. Distribution of wild-type porcine reproductive and respiratory syndrome virus (WT-PRRSV) detections by weeks (wks) post placement into the wean to finish sites among all sequences classified as wild-type (n = 36) sequences. Number at the top of each bar indicates the number of sequences.

highlighted as references. WT-PRRSV was confirmed in seven out of 10 (70%) production companies and in twenty three out of the 54 (43%) vaccinated herds.

One hundred and three sequences were classified as vaccine-like sequences (avg Ct 29.6). The vaccine-like sequences compared to their respective vaccine reference sequence had an average of 0.56% nucleotide differences. The vast majority of the vaccine-like sequences were obtained at the 2nd and 3rd sampling events, with 47 and 32 of sequences, respectively. There were fewer vaccine-like sequences obtained at the last two sampling events (6 and 3 sequences, respectively). In contrast, detection of WT-PRRSV sequences was highest (12 sequences) at the 4th sampling event. However, there were no differences in the proportion of WT-PRRSV detected between weeks post placement ($p = 0.087$) (Fig. 4).

3.4. ELISA and PCR results

Eighty nine percent of samples (2215 out of 2477) tested positive by ELISA and 44% (1094 out of 2475) tested positive by RT-PCR. Table 3 shows results for ELISA S/P ratios and PCR Ct values from all samples from the 60 WTF herds included in the analysis.

The median ELISA S/P ratio for samples from vaccinated herds with WT-PRRSV (n = 996) was 7.8 and median PCR Ct value (n = 995) was 38.7. Median ELISA S/P ratio for samples from vaccinated herds with no WT-PRRSV (n = 1307) was 6.8, and median PCR Ct value (n = 1309) was 37. There were differences between sampling points in both, ELISA and PCR Ct values in vaccinated herds with and without detection of WT-PRRSV ($p < 0.001$, Mann-Whitney test). Herds in which WT-PRRSV was detected had higher ELISA S/P ratios at 16, 20 and 25 weeks post placement ($p < 0.001$) and lower PCR Ct values at 12, 16 and 20 weeks post placement ($p < 0.001$) compared to herds with no WT-PRRSV detected (Fig. 6).

3.4.1. Survival analysis for WT-PRRSV detection

To further investigate the time of WT-PRRSV introduction into WTF herds, a right censored survival analysis using the Kaplan-Meier method

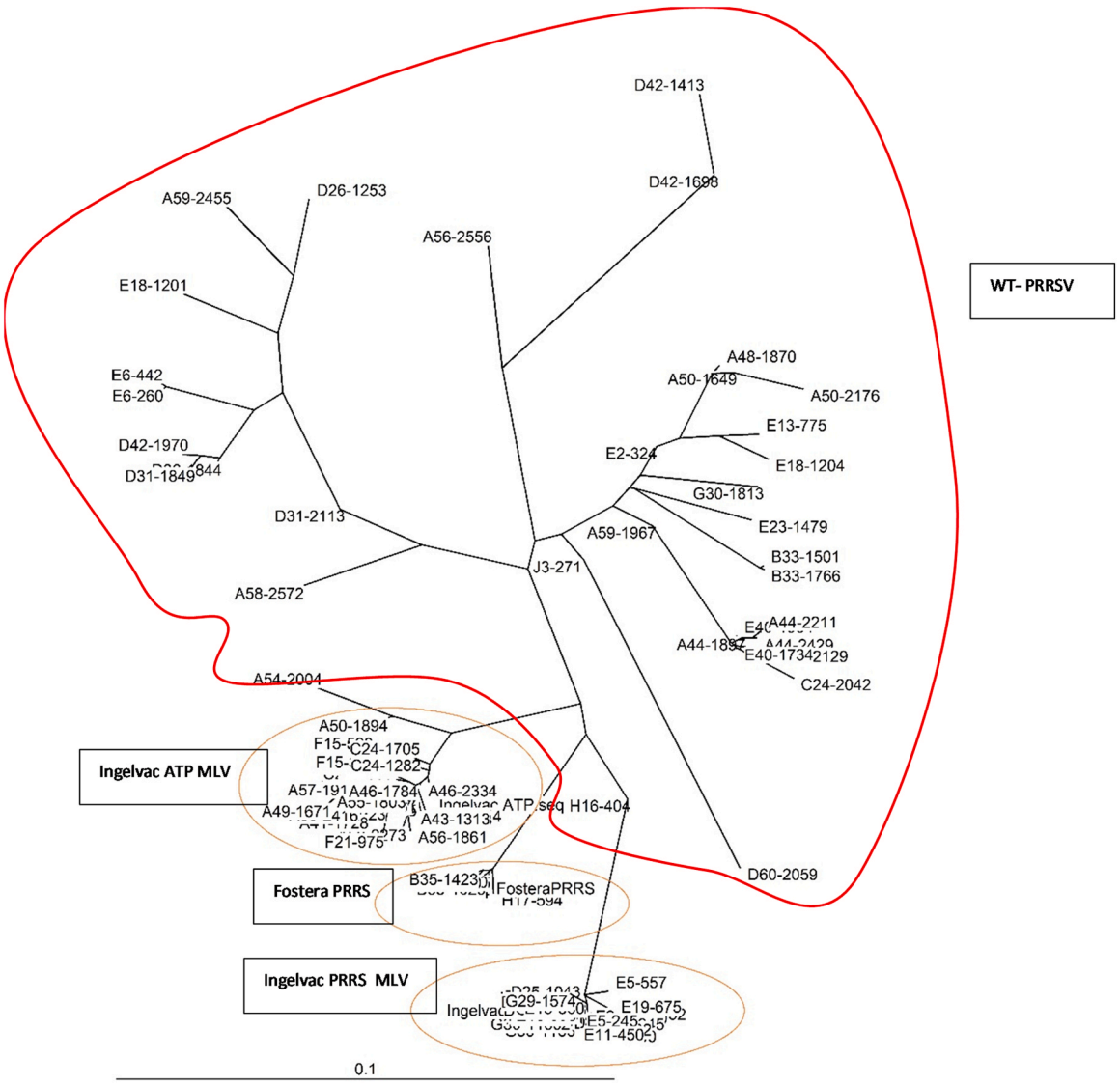

Fig. 5. Radial phylogenetic tree of sequences from production companies obtained during the study. Vaccine reference strains are shown in yellow circles and wild-type porcine reproductive and respiratory syndrome virus (WT-PRRSV) are shown in the red circle.

Table 3
Mean, standard deviation (SD), minimum, maximum median, 25% (Q1) and 75% (Q3) percentiles values of samples tested by ELISA (S/P) and PRRS RT-PCR (Ct), and number (%) of positives.

Variable	n	Mean	S.D.	Min	Max	Q1	Median	Q3	Positive (%)
PRRSV ELISA (S/P)	2477	6.16	3.22	-0.03	13.30	4.77	6.82	8.17	2215 (89%)
PRRSV PCR (Ct)	2475	39.53	6.61	24.67	45.00	32.75	45.00	45.00	1094 (44%)

was implemented considering the first sampling point when WT-PRRSV was detected in a given herd. Mean time to first WT-PRRSV detection was 21 weeks post-placement (95% CI 19, 22). The probability of no infection with WT-PRRSV in WTF herds was 53% during the whole observation period.

3.5. Mortality analysis

Mortality rates (cumulative incidence of deaths) as percentage of weaned pigs placed obtained from 58 herds were compared between sites with WT-PRRSV detected (n = 25) and sites without WT-PRRSV detection (n = 33). Mortality rates were 6.3% in sites with WT-PRRSV and 4.9% in sites where WT-PRRSV was not detected (p = 0.19). Percentage mortality in vaccinated herds (n = 52) compared to non-vaccinated (n = 6) herds regardless of WT-PRRSV detection was 5.7% vs. 3.97%, respectively (p = 0.160). For those herds that were vaccinated and had WT-PRRSV (n = 23), mortality was 6.5% compared to 5% for vaccinated herds where WT-PRRSV was not detected (n = 29) (p = 0.07) (Fig. 8).

Mortality (cumulative incidence) for herds that had WT-PRRSV detected (n = 25) is shown in Table 5. In general, herds with the earliest detection of WT-PRRSV had higher mortality rates than those where WT-PRRSV was detected late in the growing period.

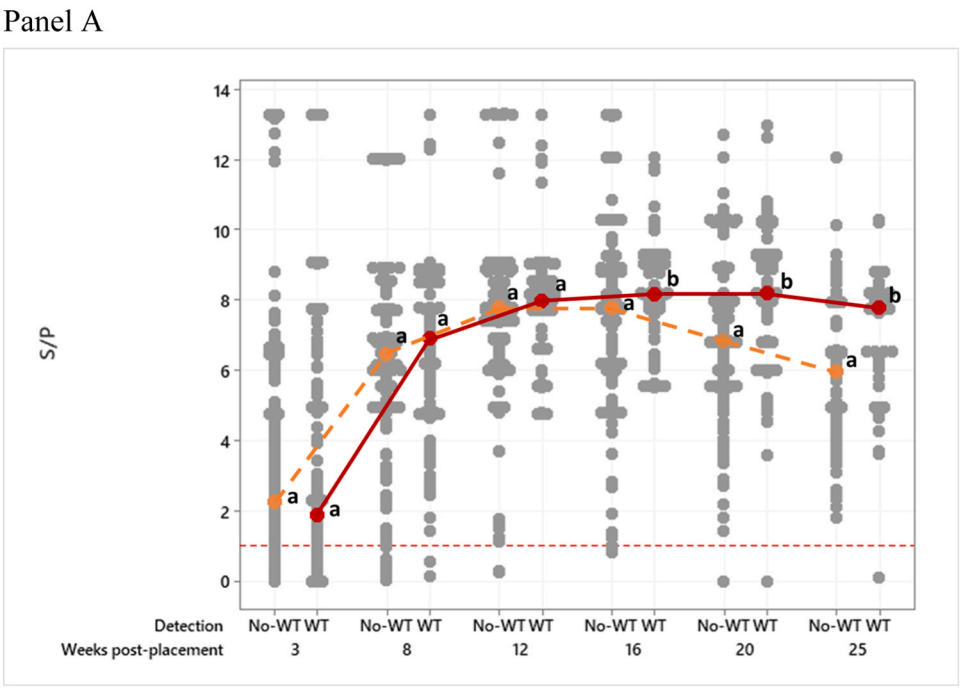

Fig. 6. Dot plot of porcine reproductive and respiratory syndrome (PRRS) ELISA sample to positive (S/P) (Panel A) and RT-PCR cycle threshold (Ct) values (Panel B) from vaccinated wean to finish (WTF) herds with and without wild-type PRRSV (WT-PRRSV) detection by weeks post placement. The orange points indicate median values for herds where only vaccine-like sequences were detected. The red points indicate data median values for herds where WT-PRRSV was detected. Different superscripts (a,b) indicate statistically significant differences (p value < 0.05) between sites with and without WT-PRRSV detection.

4. Discussion

More than half of the cost of PRRS occurs in growing pigs and although growing pigs play an important role in the overall PRRSV epidemiology, there is limited information on disease incidence, infection patterns and general understanding of when and how PRRSV infections occur in growing pigs. In this study, we evaluated the incidence and temporal distribution of PRRSV infections throughout the growing period, and the mortality observed in the herds affected with WT-PRRSV. We found that incident WT-PRRSV infections of groups of pigs placed from PRRS negative or positive stable sow farms (i.e., presumed without active PRRS infections in the breeding herds) were common in areas of medium to high pig density. This was observed despite the fact that most (90%) of the WTF herds enrolled in the study were vaccinated. Although WT-PRRSV infections were identified throughout the growing period, they tended to be more common in the latter periods of the growing stage. Our findings indicate that new introductions of WT-PRRSV into WTF herds are common, and that activities happening during the growing stage maybe responsible for these introductions of WT-PRRSV.

Approximately half of the WTF herds in our study reported new WT-PRRSV infections. Our value is consistent with previous reports of 39% (Holtkamp et al., 2013) and 55% (Gebhardt et al., 2020). However, incidence is likely to be higher given that in our study we excluded herds stocked with positive weaned pigs and considering that this was an observational study, a risk of bias should be taken into consideration. For example, we may have underestimated the overall positivity rate of WT-PRRSV infections given that PRRSV vaccination was common, and the diagnostic algorithms used to differentiate vaccine-like strains from wild type ones may not have been sensitive enough to detect all

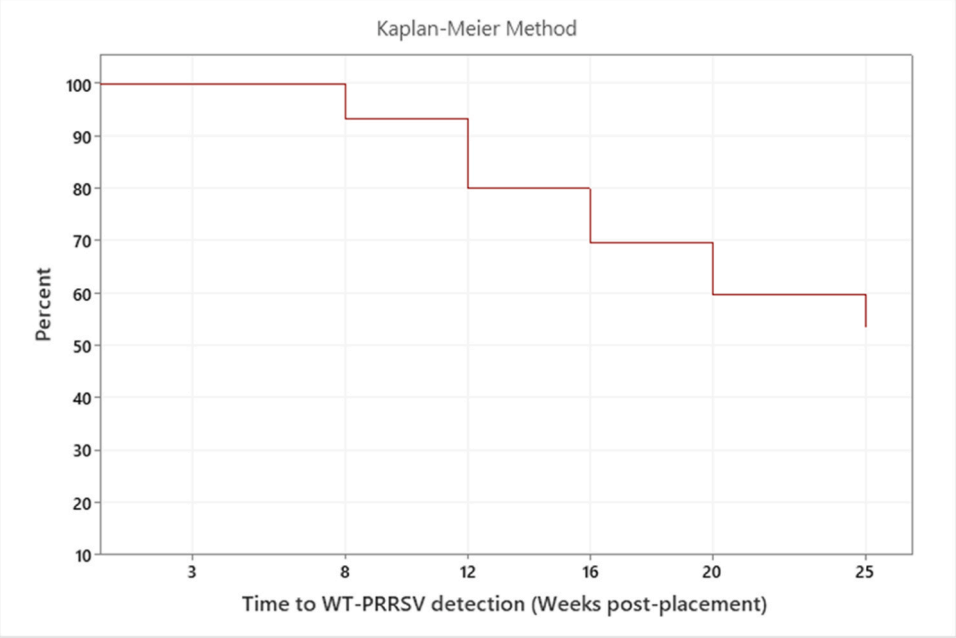

Fig. 7. Kaplan-Meier survival plot for time to first wild-type porcine reproductive and respiratory syndrome virus (WT-PRRSV) detection.

Table 4
Survival analysis for time to first wild-type porcine reproductive and respiratory syndrome virus (WT-PRRSV) detection.

Weeks post placement	WTF herds at risk	Herds with WT PRRSV detection	Survival Probability	Standard error	95% Normal CI
8	60	4	0.93	0.03	0.87 – 1.00
12	56	8	0.80	0.05	0.69 – 0.90
16	46	6	0.69	0.05	0.57 – 0.81
20	35	5	0.59	0.06	0.46 – 0.72
25	19	2	0.53	0.07	0.39 – 0.67

WT-PRRSV infections. Nevertheless, our results indicate that WT-PRRSV infections in growing pigs are common and support the fact that growing pig herds are epidemiologically important reservoirs for WT-PRRSV.

Co-circulation of WT-PRRSV and vaccine viruses was common. We reported that new infections of WT-PRRSV were distributed throughout the growing phase, and although we did not find significant differences between weeks post placement, we observed WT-PRRSV detections more often in the mid to end of the finishing phase compared to the early phase. In contrast, the peak of RT-PCR positive samples occurred sooner at approximately 8 weeks post placement likely due to the use of vaccination around weaning age. The prevalence of RT-PCR positive samples in herds where only vaccine-like PRRSV was detected decreased significantly, to near zero, by the end of the growing period while prevalence remained elevated in herds with WT-PRRSV infections. When we analyzed the distribution of PRRSV using ORF5 sequencing data, most of PRRS MLV sequences were observed around 8 and 12 weeks post-placement and only two herds had MLV-like virus at 25 weeks post-placement (Supplemental material). On the other hand, WT-PRRSV sequences were mostly detected between 12 and 16 weeks post-placement and remained common to the end of the growing period. The mechanism of PRRSV persistence at the population level is not fully understood, but co-circulation of distinct strains and vaccination with MLV vaccines (Chaudhari and Vu, 2020) may contribute to WT-PRRSV persistence and the emerging of potential recombinant viruses (Bian et al., 2017; Wang et al., 2019). Because of the sequencing strategy employed in this study, we did not consider the analysis of recombinant strains between WT-PRRSV and vaccine viruses. Recombination is known to occur thus, good vaccination practices and biocontainment measures should be considered when vaccinating grow-finish pigs.

Our results indicate the importance of new infections in growing pigs, the need to understand when and how they happen and that MLV-like strains can persist under field conditions longer than previously reported in experimental studies (Chaudhari and Vu, 2020). However, identifying possible sources of virus introduction requires further analysis and this was outside the scope of this study.

WT-PRRSV was widespread within the production companies of the study. Companies had an average of 3 genetically distinct viruses circulating in the herds and up to 6 distinct PRRS viruses in one of the companies. There was also evidence that different production companies had genetically similar WT-PRRSV (<2% ORF5 nucleotide difference), which suggest the transmission of WT-PRRSV between herds of different production companies or a common origin of the WT-PRRSV. Investigations of any links between these production companies was out of the scope of the study and therefore was not pursued. Overall, our findings confirm that growing pigs are reservoirs of multiple genetically distinct viruses, and that efforts to control PRRSV should be regional.

Risk factors for new introduction of WT-PRRSV infections were not investigated in this study. The few studies published in growing pigs have pointed out that factors like transportation, feed delivery, pig density and connectivity between sites (Arruda et al., 2016, 2017) are important factors for virus spread and that interventions like vaccination (Linhares et al., 2012, Galvis et al., 2022) and biosecurity practices (Galvis et al., 2022) can help reduce virus transmission in growing pigs. Our study supports the need to continue studies to understand transmission of WT-PRRSV in growing pigs.

To better understand the dynamics of PRRSV infections in growing pigs and pin point when WT-PRRSV infection may have occurred, we combined the detection of both antibodies and virus by ELISA and RT-PCR, respectively, and compared infection dynamics to those occurring in herds with MLV-viruses only. Using fixed spatial sampling every 4 weeks, we were able to identify different patterns of antibodies and virus circulation in vaccinated pigs from WTF sites with or without WT-

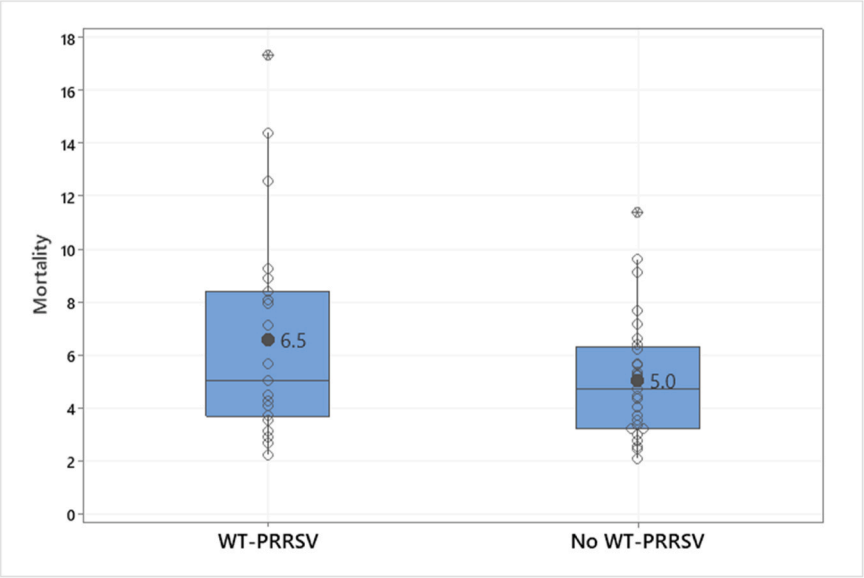

Fig. 8. Cumulative incidence of wean to finish mortality for vaccinated herds with and without wild type porcine reproductive and respiratory syndrome virus (WT-PRRSV) detected (p = 0.07). Each dot represents an individual herd.

Table 5
Median percentage closeout mortality by when wild-type porcine reproductive and respiratory syndrome virus (WT-PRRSV) was detected for the first time in the wean to finish herds.

Weeks post-placement	No. of herd with WT-PRRSV detected for the first time	Median	Min	Max
8	4	10.48[a]	5.65	14.40
12	9	4.47[ab]	2.95	9.26
16	5	4.25[ab]	3.11	17.35
20	5	2.88[b]	2.22	8.89
25	2	4.55[ab]	4.06	5.04

Different superscripts indicate statistically significant difference (p < 0.05).

PRRSV detection. Levels of PRRSV antibodies measured by S/P ratios were significant at 16 weeks post placement with antibodies remaining elevated in WT-PRRSV infected herds. In herds with only MLV-viruses detected, antibody levels followed an expected decay curve after the peak detection although they never reached negative levels. Analysis of ELISA S/P ratio dynamics proved valuable in the time series analysis. Differences in PRRSV infection levels measured by RT-PCR preceded those observed by ELISA and differences between herds with and without WT-PRRSV were observed at 12 weeks post placement and throughout the end of the growing period. The combined testing of measuring antibody and virus levels in vaccinated WTF herds supports the main outcome of the study that WT-PRRSV circulation is more prevalent in the mid to late growing period. Furthermore, our approach to sample herds every 4 weeks following a spatial-fixed approach to sample collection, appeared robust to detect differences in infection patterns using either RT-PCR or ELISA tests and was in agreement with recommendations by others (Rotolo et al., 2017; Henao-Diaz et al., 2020) that suggested that the approach of conducting on-going site-level surveillance based on fixed time intervals was preferable to collecting many samples sporadically and/or randomly.

As part of this study, we were interested in evaluating the impact of WT-PRRSV infections on mortality. We obtained closeout data from most of the enrolled WTF herds and our analysis supported the general observation that herds with WT-PRRSV had higher mortality levels than sites where WT-PRRSV was not detected. Also, we were not able to investigate the causes of mortality which may explain why there were no differences between vaccinated and non-vaccinated herds. When we analyzed mortality data by time of initial WT-PRRSV detection, we described higher mortality levels in sites where WT-PRRSV was confirmed earlier than in those where it was detected later in the growing stage. This observation suggests that pigs infected earlier are at higher risk for prolonged duration of infection and is in agreement with a recent study by Moura et al., (2022). Overall, our study supports the need to develop effective biosecurity interventions throughout the growing period in order to reduce or avoid new WT-PRRSV introductions and mitigate the economic impact of PRRSV infections.

Our study has some limitations. First, herds were not selected randomly but rather were selected by convenience based on willingness to participate in the study. Given that PRRSV is widespread in the US swine industry and that production companies employ similar disease management practices, we think differences are due to those practices rather than the collaborative relationships between the producers and the researchers. Second, use of vaccination with MLV products was widespread. Although we would have preferred to include more non-vaccinated herds in order to evaluate the effects of vaccination, use of vaccination was extensive and it was impossible to enroll more non-vaccinated herds. Vaccine use likely reflects producers' attitude to control WT-PRRSV infections and willingness to decrease the impact of disease on production costs. Use of MLV vaccination, however, introduced diagnostic challenges to our approach to differentiate PRRSV vaccine strains from WT-PRRSV. Given that only a subset of samples (lowest Ct (if < 33) at each sampling event) could be sequenced, it is likely that we have underestimated the overall incidence of WT-PRRSV introductions. For example, it is plausible in some herds that vaccination could have provided enough protection to limit replication and transmission of WT-PRRSV below the limit of detection of our sampling protocol using oral fluids.

5. Conclusion

Our results indicate that new WT-PRRSV introductions in WTF pigs originating from PRRSV negative or stable herds are common in swine dense regions of the US Midwest. Given that most of the new infections happened towards the middle to end of the growing period suggests that infections were not random but rather associated to specific practices taking place during the growing period. However, it was outside the scope of this study to evaluate whether biosecurity practices were associated to PRRSV infections. Lastly, the increased mortality observed

in growing pigs because of WT-PRRSV infections should serve as a motivation to producers to mitigate new PRRSV introductions. However, future studies are needed to evaluate what specific practices are associated with the introduction of WT-PRRSV into growing pigs so that specific changes to production practices to mitigate the impact of PRRS are made since vaccination only is not enough. Furthermore, we provided evidence that co-circulation of vaccine and WT-PRRSV viruses is common which is an important consideration when implementing surveillance programs in growing pigs. Overall, our study raises awareness of PRRSV infections in growing pigs and the need to do more to prevent them.

Declaration of Competing Interest

All authors have reviewed the manuscript and declare that they have no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.105976.

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