RESEARCH REPORT
SWINE HEALTH

Title: "Assessing within-herd PRRS variability and its impact on production parameters", **NPB #18-167**

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Industry Summary:

The objective of this study was to describe porcine reproductive and respiratory syndrome virus (PRRSV) variability within farms using farms of different demographic types (breeding and growing pig herds) and PRRS management strategies (vaccination, active outbreaks) over a long-term time span; and to investigate whether PRRSV variability has an impact on health and production outcomes.

We enrolled five farms across four U.S. states for this study and followed them for a period between 6 and 12 months. We took several sample types, focusing on those that are commonly used by veterinarians and farm personnel (processing fluids for breeding herds and oral fluids for growing pig herds), and a non-traditional sample type (tonsil scrapings), and submitted the samples to RT-PCR testing and both ORF5 and whole-genome (WGS) sequencing.

Our key results were that on ORF5 sequencing, up to three different PRRSV lineages could be found in a single sampling event (month) for single farms. Additionally, we reported different PRRSV lineages detected from month-to-month for most herds. We also reported that tonsil scrapings appeared to be a useful sample type for PRRSV detection in growing pig herds (regardless of vaccination status), but not for breeding herds, in which processing fluids appeared to be best. We were not able to obtain WGS from our samples.

The take-home message from our study was that that sequencing results based on only one sequenced sample per site for a given point in time should be interpreted with caution; especially when trying to make conclusions regarding source of a new outbreaks and the possibility of PRRS re-emergence in swine herds. Sequencing multiple samples in a sampling event would likely give a more comprehensive picture of PRRSV diversity within swine herds.

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Key Findings:

- Tonsil scrapings can be used as a PRRSV monitoring tool in growing pig populations and can be complementary to oral fluid PRRS detection via PCR under field conditions
- More than one PRRSV lineage can be found in swine herds (both breeding and growing pig herds) in a single sampling event
- More than one PRRSV lineage/ variant can be found in swine herds (both breeding and growing pig herds) across sampling events- within 1 to 6 months
- Important PRRS management decisions and conclusions (e.g. PRRS re-breaks versus PRRSV new introductions) should be based on sequencing information from more than a single sample

Keywords:

PRRSV management, PRRSV sequencing, PRRSV diversity, tonsil scrapings, PRRSV lineages

Scientific Abstract:

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the major health and economic concerns in the swine industry worldwide. Even though molecular diagnostic tools for PRRSV detection and classification have improved dramatically over the past years, the fact that this virus mutates rapidly combined with testing costs makes it difficult to understand and track PRRSV diversity in pig farms. The lack of understanding of PRRSV variability in swine herds in turn complicates outbreak investigations and prevention strategies in the field.

The objective of this study was to describe the genetic variability of PRRSV in swine farms with different production types (breeding and growing pig herds) and PRRS infection statuses (new outbreaks, vaccinated farms, "natural" PRRSV exposure). Five US swine herds (three farrow-wean herds namely Farms 1, 2 and 3, and two growing pig herds namely Farms 4 and 5) were enrolled in this project and followed up for 6 months to one year. Processing fluids, oral fluids and tonsil scrapings were sampled approximately once per month.

The study results suggested that PRRSV was more likely to be detected in processing fluids compared to tonsil scrapings (odds ratio (OR) = 3.86) in breeding farms; whereas oral fluids were outperformed by tonsil scrapings (OR = 0.26) in growing pig farms.

In addition, ORF5 sequencing in PF, OF, and TS samples validated the simultaneous detection and temporal dynamic of PRRSV lineages. Phylogenic analyses on the PRRSV ORF5 gene in processing fluids identified the presence of multiple PRRSV (genetic identity > 98%) classified into different lineages in two breeding farms within and between sampling events. Both field lineages and modified live vaccine lineages (lineage L5) were observed in oral fluid and tonsil scraping samples for both growing herds.

This study showed moderate correlations between PRRSV detections and reproductive performance in breeding herds. PRRSV detection was indicative of a temporary surge in stillborn and mummified piglets in Farm 2 and 3 of the farrow-wean farms ((PF: rho = 0.86, p = 0.003; TS: rho = 0.89; p = 0.001) and mummified fetuses (PF: rho = 0.92, p < 0.001; TS: rho = 0.92; p < 0.001) and (stillborn: rho = -0.82, p = 0.002; mummified: rho = 0.63, p = 0.04) as well as higher pre-weaning mortality in Farm 3 (rho = -0.65, p = 0.03).

Additional research is needed to attempt whole genome sequencing, which was not successful in the current study with the sample types collected herein.

Introduction:

It has been well reported that the porcine reproductive and respiratory virus is very dynamic in nature, and because it is an RNA virus, it changes rapidly and continuously creating numerous local variants (Goldberg et al., 2003). This observed genetic, antigenic, and clinical heterogeneity is one of the main obstacles to understand, prevent and control this disease in swine herds.

Even though PPRSV diversity is recognized, current sampling and diagnostic strategy schemes used by field veterinarians (i.e. sequencing of part of the PRRSV genome using one sample per swine site) have not been well evaluated for a highly mutating RNA virus such as PRRSV. This becomes especially important when considering the conclusions and consequences we are making on a regular basis. For example, determining that a PRRSV sequenced after an outbreak (using part of the genome, the open-reading frame 5 [ORF5]) is a new virus for the specific farm, a genetic difference of at least 2-4 % compared to a previously sequenced strain needs to be obtained. This practice normally indicates this is a new virus for the farm, and this has been a useful guide to indicate relatedness of two virus isolates (Murtaugh, 2012).

The main molecular method currently widely used for PRRSV outbreak investigations is the sequencing of the ORF5 gene, which has been widely used to predict how isolates are correlated and to characterize existing diversity within a farm or a system. However, the ORF5 gene only represents approximately 4% of the whole PRRSV genome (Murtaugh, 2012), and single nucleotide polymorphisms (SNPs) have not only been restricted to ORF5, but to other parts of the PRRSV genome.

However, the identification methodology of new strains often leads to frustrating and lengthy reviews of biosecurity practices as an attempt to identify “what went wrong” and possible areas for improvement, and may even lead to more extreme and costly decisions such as filtering a barn in order to reduce the likelihood of area spread. In reality, the new strain may have originated from a resident strain within the farm. So far however, we have not been able to understand PRRSV within-farm diversity at the same time as discriminating between resident and newly introduced PRRSV variants at the farm level.

With the advent of next generation sequencing (NGS) and increasing availability and affordability of whole genome sequencing, we now have advanced tools for better understanding of PRRSV variation (commonly referred to as quasispecies), its prevalence, persistence and re-emergence at the herd level. WGS is a promising emerging tool that may provide higher depth of information to better understand virus evolution, co-infection events, and detection of new PRRSV quasispecies.

The coexistence of multiple genetic variants within farms and over time has been previously explored, but only using ORF5 gene sequencing. A study conducted in Quebec reported that 78% of the herds with multiple laboratory submissions were identified with different PRRSV strains, and often within a one-year period (Larochelle et al. 2003). Another study conducted in Illinois showed that multiple variants of PRRSV could exist simultaneously on farms and even within individual animals during natural infection (Goldberg et al., 2003). Furthermore, a case study reported the coexistence of three genetically diverse groups of PRRSV in a 1,750 chronically infected sow farm over a period of one year (Dee et al., 2001), with ORF5 sequence variance ranging from 5.8 to 11%. Finally, a North Carolina case report described distinct PRRSV within farms from two different systems, and they also concluded that more clinical losses may occur due to PRRS when a multiplicity of PRRSV variants are present (Roberts, 1999).

Under field conditions, the rate of co-circulation of different PRRSV variants and the implication for PRRS management decisions has often been discussed but has not been studied extensively using WGS. We therefore can conclude that it is timely for a study to address PRRSV variation and genomic evolution in swine production and clarification of re-emerging PRRSV outbreaks.

Objectives:

Objective 1: Describe PRRSV quasispecies within individual farms using a sample of farms of different demographic types and PRRS management strategies over a one-year time span; and to investigate whether PRRSV variability has an impact on health and production outcomes.

Objective 2: Investigate and compare the use of WGS and different ORFs to determine the best predictor to identify and relate viruses within swine herds

Objective 3: Correlate PRRSV variants with production and disease metrics.

Materials & Methods:

Swine farms were recruited for this long-term longitudinal study. The recruitment process required a combination of in-person farm visits and e-mail dissemination of enrollment opportunity.

Five US swine herds (three farrow-wean herds, and two growing pig herds) were enrolled in this project, which are described in detail in Table 1. For the breeding herds, farms were either recently infected (active outbreak), actively vaccinated (using an MLV vaccine), or "naturally" infected (no vaccine used); while for the growing pigs herds, farms were either recently infected (active outbreak), or actively vaccinated (using an MLV vaccine).

After farms were identified and enrolled, sampling protocols were designed based on each farm's demographics and provided farm maps and shared with herd veterinarians and personnel. Sampling began between February of 2019 and April of 2019, depending on the farm, with the goal to complete 12 months of sampling in each farm.

Tonsil scraping samples were collected monthly from all herds (on an individual animal level); processing fluids were obtained from piglet processing batches within the three breeding farms, and pen-based oral fluids were collected in the two growing pig farms using fluids extracted from ropes attached to the pen walls.

Table 1. Description of farms enrolled in the study

Farm	Type	PRRS status	Date of last outbreak	Location	Herd size	Start of sampling
1	Farrow-wean	Partially immune; no longer vaccinating or exposing	Aug 2018	Ohio	5,000 sows	May 2019
2	Farrow-wean	Negative (last break Nov 2012) with new break (vaccinated and inoculated); filtered	Jan 2019	Iowa	6,000 sows	February 2019
3	Farrow-wean	Vaccinated (at least 2X year with MLV)		Oklahoma	2,500 sows	February 2019
4	Wean-finish	Negative with new break; not vaccinating	March 2019	North Carolina	3,550 pigs	April 2019
5	Finisher	Vaccinated with MLV (vaccinated at processing)		North Carolina	2,800 pigs	April 2019

During the months of June and September of 2019, and July and August of 2020, samples were tested using qRT-PCR and RNA was extracted in preparation for whole genome sequencing; under Dr. Declan Schroeder's laboratory (Co-I in the proposal).

Objective 1:

Viral RNA extraction and reverse-transcription quantitative PCR (RT-qPCR) were conducted for all samples, with the sample positivity threshold set at quantification cycle (Cq) of ≤ 37. The PRRSV detection for each specimen was described using the percentage of positives among all samples collected on the sampling event. Generalized linear models were built in R separately for each herd type (breeding and growing pig herds) to investigate the effect of specimen type, sampling season, and farm PRRS infection status on PRRSV positivity (as determined by RT-qPCR results). Statistical significance was declared at $P < 0.05$.

PRRSV genetic diversity was evaluated by sequencing the ORF5 region of samples with Cq < 31, performed by University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL) collaborators (Co-Is Mor and Schroeder). For phylogenetic analysis, the type of PRRSV were first determined by aligning identified sequences to PRRSV type 1 (Lelystad; NC_043487.1) and 2 (VR-2332; GenBank ID EF536003.1) prototypes (Paploski et al., 2019). Secondly, sequences were further aligned to 690 ORF5 sequences anchoring 16 PRRSV type 2 lineages and sub-lineages (Paploski et al., 2019; Paploski et al., 2021). The presence of different lineages indicated the co-circulation of multiple PRRSV strains. PRRSV were defined as different when the percent identity was < 98%.

Objective 2:

Whole genome sequence was attempted using a validated Oxford Nanopore Technologies (ONT) platform as described in Schroeder et al., 2021. RNA was extracted using manufacturer's instructions from MagMAX™-96 Viral RNA Isolation (Applied Biosystems by Thermo Fisher Scientific) from field collected tonsil scrapings, processing fluids and oral fluids as described above. cDNA libraries were generated using the SMARTer Universal Low Input RNA kit for first-strand synthesis, barcoded using Illumina & MinION barcodes, and targeted PRRSV genomes using a custom designed universal PRRSV specific primer and the PrimeStart® GXL polymerase for second-strand synthesis (Takara Bio USA Inc.). The ONT libraries were prepared using the ligation SQK-LSK109 sequencing kits. All libraries were individually barcoded, where appropriate, and sequenced using the high-accuracy base calling model with a minimum QScore of 7 set on an ONT MinION sequencing platform using multiple FLO-MIN106 R9 flow-cells. The reads obtained were QC checked and analyzed using the Epi2ME WIMP blast software.

Objective 3:

Production data was requested and obtained in the second semester of 2020, when results were available for presentations with project participants. The correlation between production data and PCR positivity was examined using Spearman's rank correlation coefficient (Spearman's rho) using R 4.0.4 (R Core Team, 2021).

Results:

Objective 1:

Sample collection was completed anywhere between 6 and 12 months into the project depending on the farm, which resulted in 6 to 12 samplings successfully collected (Table 2). All collected samples were sent directly to the University of Minnesota and stored in Dr. Sunil Kumar's laboratory (Co-I in the proposal).

Table 2. Sampling scheduling and qPCR/ RNA extraction progress for enrolled farms. Orange cells show completed sampling, qPCR, and RNA extraction. Blank cells show projected future sampling months.

	Sampling											
Farm	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1*	05/19	05/19	06/19	07/19	08/19	09/19	-	-	-	-	-	-
2	02/19	03/19	04/19	05/19	06/19	07/19	08/19	09/19	10/19	-	-	-
3	02/19	03/19	04/19	05/19	06/19	07/19	08/19	09/19	10/19	11/19	02/20	03/20
4	04/19	05/19	06/19	07/19	08/19	09/19	10/19	11/19	12/19	02/20	-	-
5	04/19	05/19	06/19	08/19	09/19	10/19	11/19	12/19	01/20	03/20	-	-

*Farm 1 had one sampling early in May (05/06/2019), and one later in May (05/13/2019)
Orange shades demonstrate fully complete sampling (16 total samples submitted), grey shades represented missing samples (8-12 samples submitted). Dashes represent no sampling event.

Tonsil scraping samples (n = 343) were collected monthly from all herds (individual animal level); processing fluids (n = 216) were obtained from piglet processing batches within the three breeding farms, and pen-based oral fluids (n = 125) were collected in the two growing pig farms.

PRRSV was detected by RT-PCR in approximately 25% of tonsil scrapings, 22% of processing fluids, and 25% of oral fluids throughout the study period. The Cq values from the samples were overall relatively high, precluding several samples from being sequenced using the ORF5 and precluding all samples to be whole genome-sequenced (Figure 1)

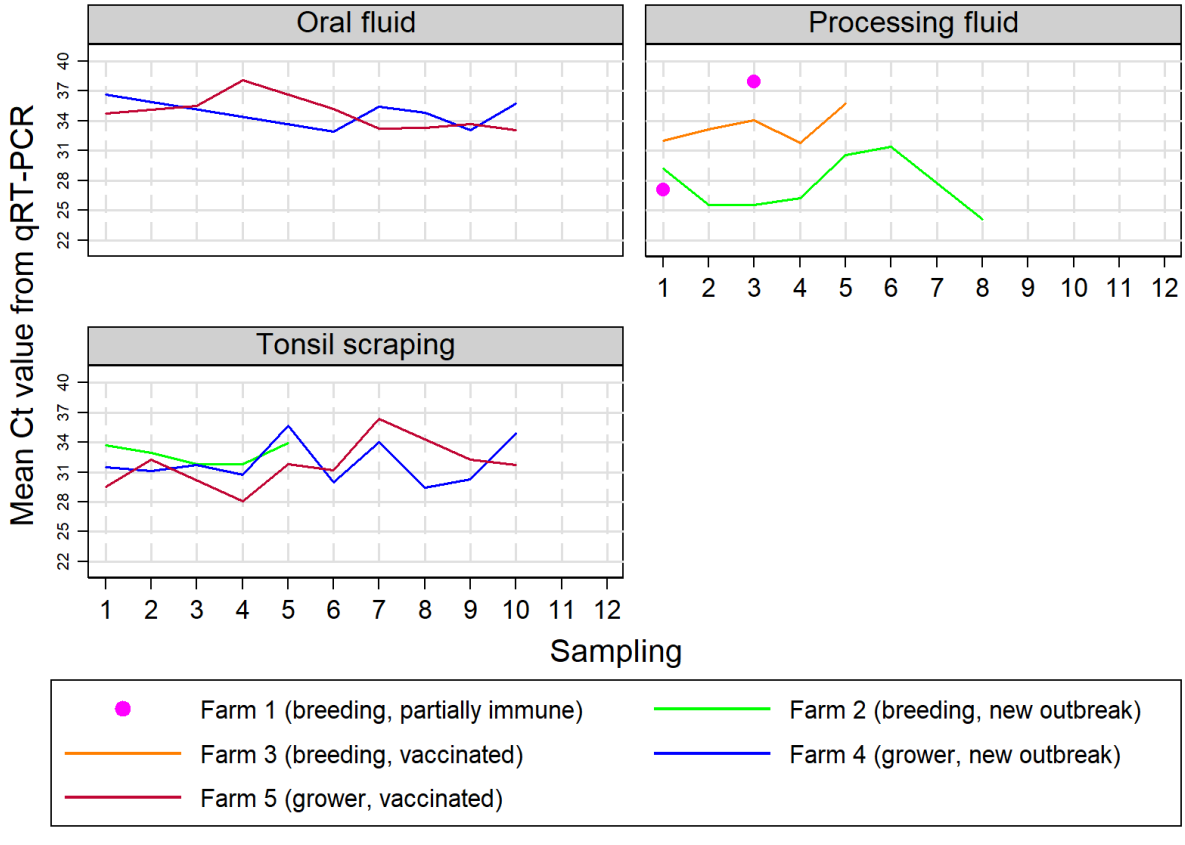


Fig. 1. Mean Ct values from qPCR results for all farms enrolled in the project over the sampling events; and for the three different sample types (tonsil scrapings, oral fluids and processing fluids). Please note that farms 4 and 5 only had 10 sampling events; while farms 1, 2 and 3 completed a number of 6, 9 and 12 sampling events, respectively. Also to be noted, processing fluids are only taken in breeding herds and oral fluids, growing pig herds.

Results from statistical models suggested that PRRSV was more likely to be detected in processing fluids compared to tonsil scrapings (odds ratio (OR) = 3.86) in breeding farms; whereas oral fluids were outperformed by tonsil scrapings (OR = 0.26) in growing pig farms.

PRRSV ORF5 sequences were successfully identified from 34 PF (15.7%), 7 OF (5.6%), and 12 TS (3.5%) samples. All ORF5 sequences were classified into PRRSV type 2.

Phylogenic analyses on the PRRSV ORF5 gene in processing fluids identified the presence of multiple PRRSV (genetic identity > 98%) classified into different lineages (L1H, L1A, and L8) in two breeding farms within and between sampling events. Both field lineages and modified live vaccine lineages (lineage L5) were observed in oral fluid and tonsil scraping samples for both growing herds.

Objective 2:

For the RNA samples, 16 /93 samples tested did not yield quantifiable levels of cDNA (Fig 2). However, we proceeded to barcode all the cDNA and the total sequencing of all samples produced 108 thousand reads, equating to 55 million bases. Disappointingly, no positive matches to PRRSV were observed. The main matches were to bacteria (43%) and associated phages. The sequencing effort did however detect the Porcine Astrovirus and the endogenous retrovirus.

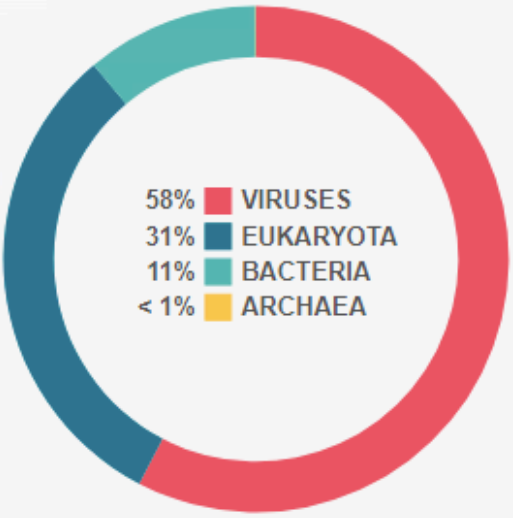
Sample ID	Farm ID	Sampling #	Sample type	RNA ng/µL	PRRSV RTPCR Results (Ct)	Illumina BC	Minion BC	cDna Conc
6	1	2	processing fluid	95.882	Negative	1	1	25.2
7	1	2	processing fluid	177.082	Negative	2	1	14.6
205	1	1	processing fluid	30.036	25.17853928	3	1	16.3
206	1	1	processing fluid	107.737	23.43460655	4	1	19.5
207	1	1	processing fluid	8.701	28.64159775	5	1	NA
212	1	1	processing fluid	17.235	31.07331161	6	1	NA
17	2	1	tonsil scraping	9.881	33.90946198	2	3	21
19	2	1	tonsil scraping	7.74	37.26465988	3	3	11.9
22	2	1	tonsil scraping	8.79	33.46017456	4	3	12
24	2	1	tonsil scraping	21.556	30.08687019	5	3	6.98
25	2	1	processing fluid	100.704	Negative	1	4	11.8
26	2	1	processing fluid	84.127	28.69347	6	4	too low
27	2	1	processing fluid	26.085	37.90861511	7	4	4.42
28	2	1	processing fluid	56.563	34.44007111	8	4	3.52
29	2	1	processing fluid	56.616	29.11045074	9	4	9.3
30	2	1	processing fluid	78.675	22.676754	10	4	11.3
31	2	1	processing fluid	28.35	27.30392075	11	4	6.88
32	2	1	processing fluid	22.526	24.23418999	12	4	9.38
83	2	2	tonsil scraping	7.782	41.31433487	1	5	25
86	2	2	tonsil scraping	4.508	34.4740715	4	5	17.2
87	2	2	tonsil scraping	15.816	30.1255703	5	5	25.6
88	2	2	tonsil scraping	14.11	25.92722321	6	5	16.7
89	2	2	processing fluid	3.852	Negative	2	6	12.6
90	2	2	processing fluid	42.914	20.46434212	7	6	13.8
91	2	2	processing fluid	14.833	20.42990112	8	6	8.18
92	2	2	processing fluid	29.889	32.91738129	9	6	17.6
93	2	2	processing fluid	28.273	34.90999222	10	6	6.6
94	2	2	processing fluid	22.125	20.86336327	11	6	4.52
95	2	2	processing fluid	22.372	26.44010544	12	6	9.26
96	2	2	processing fluid	13.191	22.67074394	1	6	too low
144	2	3	tonsil scraping	7.17	Negative	3	7	too low
145	2	3	tonsil scraping	19.263	32.83003998	2	7	17.1
148	2	3	tonsil scraping	26.571	30.83559799	3	7	9.98
149	2	3	processing fluid	46.566	26.68958092	4	8	12.1
150	2	3	processing fluid	37.115	21.44708252	5	8	9.96
151	2	3	processing fluid	20.498	28.5156517	6	8	24.6
152	2	3	processing fluid	18.189	28.70712852	7	8	too low
153	2	3	processing fluid	26.492	25.63352394	8	8	9.4
154	2	3	processing fluid	26.069	23.5357933	9	8	12
155	2	3	processing fluid	26.324	26.02070427	10	8	11.3
156	2	3	processing fluid	57.837	23.92269516	11	8	5.08
174	2	4	tonsil scraping	48.424	31.79200363	12	8	12.9
181	2	4	processing fluid	56.773	29.27050209	7	10	NA
182	2	4	processing fluid	61.754	23.53647995	8	10	13.7
183	2	4	processing fluid	50.147	23.59080124	9	10	13.1
186	2	4	processing fluid	100.795	31.26740456	10	10	too low
187	2	4	processing fluid	26.019	22.51403809	11	10	too low
188	2	4	processing fluid	61.532	27.3881855	12	10	too low
33	3	1	tonsil scraping	41.099	32.45263672	1	11	40.4
34	3	1	tonsil scraping	12.66	37.94691467	2	11	22.8
40	3	1	tonsil scraping	8.032	Negative	12	11	28.4
105	3	2	processing fluid	82.796	36.61140442	3	13	7.14
106	3	2	processing fluid	125.072	31.40198517	4	13	5.98
108	3	2	processing fluid	28.903	33.57347183	5	13	8.62
110	3	2	processing fluid	24.431	31.1113987	6	13	7.52
160	3	4	processing fluid	101.928	32.07603073	7	13	9.76
161	3	4	processing fluid	106.119	Negative	8	13	too low
163	3	4	processing fluid	197.19	31.471035	9	13	too low
190	3	3	processing fluid	32.398	34.00040436	10	13	12.8
191	3	3	processing fluid	88.267	34.13872147	11	13	10.3
195	3	3	processing fluid	50.766	Negative	8	13	NA
49	4	1	oral fluids	42.376	37.6987915	12	14	12.6
52	4	1	oral fluids	36.333	Negative	1	14	14.2
54	4	1	oral fluids	35.895	34.87420731	2	14	13.6
56	4	1	oral fluids	16.415	37.52106094	3	14	9.34
57	4	1	tonsil scraping	6.917	31.61856842	4	15	too low
58	4	1	tonsil scraping	7.705	27.87714767	5	15	22.8
59	4	1	tonsil scraping	6.833	34.97358704	6	15	16.1
60	4	1	tonsil scraping	6.365	37.7838974	7	15	12.7
61	4	1	tonsil scraping	18.305	31.81447983	8	15	28.4
62	4	1	tonsil scraping	20.771	30.06654739	9	15	23.2
63	4	1	tonsil scraping	17.545	28.9949913	10	15	25.6
64	4	1	tonsil scraping	20.184	29.16703415	11	15	27
123	4	2	tonsil scraping	12.329	29.6734848	12	15	too low
124	4	2	tonsil scraping	37.9	28.67269897	1	15	25.2
125	4	2	tonsil scraping	6.86	32.93970871	2	15	too low
126	4	2	tonsil scraping	7.778	35.23816299	3	15	20.8
127	4	2	tonsil scraping	39.406	28.95728493	4	15	39.6
65	5	1	oral fluids	39.308	34.77582932	5	16	15.9
66	5	1	oral fluids	76.508	Negative	7	16	8.22
73	5	1	tonsil scraping	16.262	28.26266289	6	17	22.2
76	5	1	tonsil scraping	8.969	34.89891052	8	17	too low
77	5	1	tonsil scraping	19.152	26.48359108	9	17	17.8
78	5	1	tonsil scraping	7.129	26.98103333	10	17	15.6
79	5	1	tonsil scraping	13.449	30.75731468	11	17	22.8
80	5	1	tonsil scraping	16.104	29.9663105	12	17	0.116
130	5	2	oral fluids	39.717	Negative	7	18	15.6
135	5	2	tonsil scraping	6.545	30.50048256	1	18	32
136	5	2	tonsil scraping	38.305	27.47476987	2	18	6.54
137	5	2	tonsil scraping	36.417	30.45337868	3	18	32
138	5	2	tonsil scraping	6.797	38.42464066	4	18	too low
139	5	2	tonsil scraping	14.375	30.33787842	5	18	too low
140	5	2	tonsil scraping	5.786	36.35813522	6	18	too low

B

Fig.2. cDNA synthesis of samples for ONT sequencing

Further detailed analysis of a smaller subset of these samples, namely sample IDs belonging to Pool6 – 89 to 96, recovered 708 thousand reads equating to 298 million bases. The majority (58%) matched to viruses; however, these were once again the bacteriophage (Fig 3A). We did however detect PRRSV (Fig 3B) but not in sufficient amounts to obtain whole genome coverage.

A



READS ANALYSED
708,183

B

Taxon	Reads
Staphylococcus phage Andhra	119,019
Rakietenvirinae	6,752
Nephila clavipes virus 2	1,744
Staphylococcus phage CSA13	1,472
Klebsiella phage Marfa	896
Porcine endogenous retrovirus E	54
Bacteroides phage B124-14	33
Aeribacillus phage AP45	30
Caudovirales	18
Acinetobacter phage AbKT21phiIII	18
Arteriviridae	11
Simbu orthobunyavirus	10
Bacteroides phage B40-8	8
Aichivirus C	4
Porcine kobuvirus SH-W-CHN/2010/China	4
Porcine reproductive and respiratory syndrome virus	4
Porcine astrovirus 3	4
Vequintavirus	3
unclassified Siphoviridae	3
Porcine kobuvirus	3

Fig 3. Output from Epi2Me from Pool6. A) Percentage identity across kingdoms. B) Virus matches within Virus Kingdom

Objective 3:

Regarding PRRS variability and its relationship with production, the main findings from our study can be summarized as follows:

- Farm 1: low PRRSV detection (lineage L1H). No significant production impacts were observed in Farm 1 as the herd was not recently infected.

- Farm 2: high PRRSV detection. High pre-weaning mortality and mummified fetuses were observed in the first three sampling events, which is expected given animals were vaccinated and experiencing a recent outbreak. The PCR positivity in PF and TS samples was significantly correlated to the percentage of stillborn piglets (PF: rho = 0.86, p = 0.003; TS: rho = 0.89; p = 0.001) and mummified fetuses (PF: rho = 0.92, p < 0.001; TS: rho = 0.92; p < 0.001), respectively.

- Farm 3: moderate to low viral detection and production impacts over time were observed, this is consistent with a stable PRRSV status. Significant correlations were identified between PCR positive percentage of PF samples and all three breeding indices (stillborn: rho = -0.82, p = 0.002; mummified: rho = 0.63, p = 0.04; pre-weaning mortality: rho = -0.65, p = 0.03). Surprisingly, the ORF5 sequences were not clustered into the same lineage as vaccine strains, which indicated the circulation of wild viruses in Farm 3.

- Farms 4 and 5: considering production data was only available at the group level for these growing pig farms and considering the one-year follow-up of the study; we were not able to investigate statistical associations within these two farms. However, we did observe both field (lineage L1A) and modified live vaccine (lineage L5) lineages of PRRSV in both Farms 4 and 5 which were detected in both OF and TS samples from Farm 4.

Discussion:

The results described herein highlight the fact that multiple PRRSV lineages can be found in the same sampling event within farms, as well as over time. We also report the use of tonsil scrapings as potentially beneficial in monitoring PRRSV in growing pig populations. These findings could lead to an improvement in PRRSV diagnostic and surveillance by selecting different specimens for PRRSV detection and supporting sequencing of more PCR positive samples.

The fact that up to three different lineages (namely L1H, L1A, and L8) were identified in processing fluid samples collected on a single farm over one of the sampling events showed the simultaneous circulation of wild and vaccine strains in the herd. This is consistent with previous findings as simultaneous detection of genetically diverse PRRSV strains has been reported within a single swine farm, herd, and sample (Dee et al., 2001; Oh et al., 2019). Furthermore, a recent study investigated the co-circulation and temporal dynamics of PRRSV lineages in U.S. swine herds, has described the transition of dominant lineages from L9 to L1 after 2014 (Paploski et al., 2019).

This study showed moderate correlations between PRRSV detections and reproductive performance in breeding herds. In addition, the ORF5 sequencing in PF, OF, and TS samples validated the simultaneous detection and temporal dynamic of PRRSV lineages in consistent with previous studies and the domestic swine disease monitoring system. The limited number of ORF5 sequences obtained in OF samples may raise concerns about acquiring PRRSV genomic information in OF samples. Nevertheless, the result reported herein demonstrated the co-circulation of multiple lineages of PRRSV including wild and vaccine strains in commercial breeding and growing swine herds. This implies the deficiency and potential bias in the common practice identifying the sequences of dominant PRRSV in the field, i.e., simply submitting samples with the highest viral concentrations (lowest Cq).

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Peer-reviewed publications resulting from this grant:

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2. M. R. Campler, T.-Y. Cheng, D. Schroeder, M. Yang, S. Mor, J. B. Ferreira, A. G. Arruda. 2021. A longitudinal study on PRRSV detection in swine herds with different demographics and PRRFSV management strategies. Submitted to Transboundary Emerging Diseases, *under review*.