

Title: PRRSv detection over time in different age groups in breeding herds attempting to produce PRRSv-negative piglets at weaning: part 2: suckling pig population (NPB 18-161)

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Industry Summary:

It is clear that PRRS virus (PRRSv) has the ability to sustain infection in breeding herds at a very low prevalence. This makes it difficult to detect the virus towards the end of PRRSv-elimination programs. In other words, it is challenging to know when it is safe to bring in PRRSv-negative gilts without risk of “re-break” with the same virus. There are a couple alternatives to detect virus at low prevalence: 1) collect blood samples from a high number (150+) of pigs at weekly intervals, or 2) take advantage of ‘population-based’ sample types such as processing fluids (PF) where ‘hundreds’ of piglets are sampled at once.

The objective of this study was to characterize the patterns of PRRSV detection by PCR in processing fluids, collected at 3-5 days of age, and subsequently in family oral fluids (FOF), collected just prior to weaning (litters of 18-20 days). PF consist of serosanguineous samples derived from the process of castrating and tail docking piglets. FOF is the liquid obtained from cotton ropes exposed to lactating sow and respective due-to-wean piglets.

We followed 7 breeding herds that went through a PRRSv outbreak and were in the process of virus elimination targeting producing PRRSv-negative pigs at weaning. Those farms were monitored over time with PF and FOF. Samples were all tested for PRRSV RNA by PCR at the Iowa State University Veterinary Diagnostic Laboratory.

One of the main findings of this study was the nature of the PRRSv detection within and between weeks and rooms of a given herd. Among all farms there was intermittent weekly detection of PRRSv using PF in 15 occasions, and using FOF in 7 occasions. Within the same week, intermittent results were observed 22 times with PF and 12 times with FOF between rooms. This clearly demonstrates the crucial importance of continuously monitoring on a weekly basis, and also sampling as many rooms as possible in an attempt to minimize misclassification of farm based on the test results of a single room.

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Another important finding was the need to keep sampling farms repeatedly over time to gain confidence that PRRSv is no longer circulating. There was one farm that achieved 11 weeks of consecutive negative results with PF, and that tested PCR-positive on FOF twice. PF is a great specimen to 'screening' farrowing rooms for PRRSv. When there is failure to detect virus in PF for a few weeks, there is the need to 'double-check' the status of due-to-wean litters. FOF is a great tool in that stage.

Results provide insights for the design of improved diagnostic monitoring programs for farms attempting PRRSv elimination by suggesting a combination of PF and FOF for an increased chance to detect the virus in the suckling pig population. Also provides evidence of the intermittent nature of detection in different weeks and by room within the same week with PF and FOF. This also provides evidence that a period of more than 11 weeks of consecutive negative results with PF and FOF is necessary to establish a herd as stable for PRRSv.

Keywords: PF, FOF, PRRSv, surveillance, swine

Scientific Abstract:

One of the major challenges to successfully eliminate PRRSv from breeding herds is correctly identifying when the within-herd PRRSV transmission has been ceased, and the herd has started to consistently produce PRRSv-negative pigs at weaning. Processing fluids (PF) and family oral fluids (FOF) have been described as 'population samples' that can be used for monitoring of swine populations in breeding herds by sampling pigs at 3-5 days of age and due-to-wean pigs, respectively. However, there is still limited data on the dynamics of PRRSv detection over time by these methods in herds undergoing virus elimination.

The objective of this study was to describe the dynamics of PRRSv RNA detection by rRT-PCR in suckling piglets over time in herds undergoing PRRSv elimination in PF and FOF. Seven breeding herds attempting PRRSv elimination were followed over time with weekly sampling of PF and FOF. The PCR results of each specimen was compared and described within herd over time (weeks) and farrowing rooms, and described in an aggregated format.

Among all farms there was intermittent weekly detection of PRRSv RNA using PF or FOF in 15 and 7 occasions, respectively. Within the same week (between rooms), intermittent results were observed 22 times with PF and 12 times with FOF, which demonstrates a crucial importance of continuously monitoring on a weekly basis, by sampling as many rooms as possible in an attempt to minimize misclassification of farm based on the test results of pigs in a single room. Although one farm achieved 11 weeks of consecutive negative results with PF, testing piglets at weaning revealed the detection of PRRSV twice during that same period, which demonstrates the importance of testing the due-to-wean piglet population for a higher confidence in the results of the monitoring program.

Overall PF and FOF results matched in 73% of the occasions. FOF detected particular weeks as positive in 9.5% of the time and PF tested negative for those weeks. Also, in 17.5% of the time PF tested positive for a specific week while FOF failed to detect the same week as positive. However, when only considering matching results where the same rooms were tested by both PF and FOF, both techniques yielded the same classification for a given week in 80.7% of the times, while PF classified a week as positive and FOF as negative in 8.8% of the occasions and FOF classified as week as positive in PF as negative in 10.5% of the time.

The results of this study provide insights for the design monitoring programs for breeding herds attempting PRRSv elimination. More specifically, it supports that combination of PF (3-5 days old) and FOF (due-to-wean litters) provides an increased probability to detect the virus in the suckling pig population. The study also provides evidence of the intermittent nature of

PRRSV RNA detection in different weeks and by room within the same week with PF and FOF. Moreover, this study provided evidence that a period of more than 11 weeks of consecutive negative results with PF and FOF is necessary to establish a herd as stable for PRRSV. PF-based monitoring over time appears to be a great screening process. FOF is a great addition to the monitoring program when PCR results of PF samples start to consistently become negative for at least 8 consecutive weeks.

Keywords: PF, FOF, PRRSV, surveillance, swine

Introduction:

One of the major challenges to successfully eliminate PRRSV from breeding herds is correctly identifying when the within-herd PRRSV transmission has been ceased, and the herd has started to consistently produce PRRSV-negative at weaning.

It has been established that PRRSV has the ability to sustain infection at very low prevalence (<3%) in breeding herds undergoing virus elimination, making it difficult for veterinarians to detect it using individual pig sampling (Graham et al., 2013; Linhares, 2013; Kittawornrat et al., 2014). Thus, detecting PRRSV at low (<3%) prevalence requires cost- and time-prohibitive sample size (i.e. hundreds of samples collected at every sampling time). This has motivated the emergence of population-based PRRSV surveillance methods, including the use of processing fluids (Lopez et al., 2017a, b; Vilalta et al., 2017; Lopez et al., 2018), FOF (Yeske-Livermore et al., 2014), and others.

The feasibility of using processing fluids (PF) to detect PRRSV RNA over time in 2-5 days old piglets has been demonstrated (Lopez et al., 2017a). It has also been demonstrated the superior room-level sensitivity using 1 single PF compared to 30 serum samples. According to the Swine Disease Reporting System, 11% of sample types submitted for PRRSV PCR testing (to VDLs at ISU, UMN, KSU, and SDSU) in 2018 were PF (Trevisan et al., 2019). Also, according to a survey conducted by Dr. Corzo with the MSHMP's participants, 11 out of 21 production systems use PF for PRRSV monitoring in their breeding herds.

Even though PF is a great sample type for PRRSV monitoring, that specimen only reflects virus circulation at 3-5 days old piglets, and does not necessarily reflect PRRSV status of due-to-wean piglets (Smith et al., 2018). Thus, family oral fluids (FOF) have been described in 2014 by Lisa-Yeske (Yeske-Livermore et al., 2014), who described that each piglets spent 6 to 8 minutes in the rope. FOF is collected by hanging a rope where the sow and her respective piglets have access to it, leaving the 'family' fluids for testing. More recently, Almeida has demonstrated with a study with 72 matching sets of family fluids and serum samples from all respective piglets in the litter that FOF had 100% specificity, and 85% sensitivity to detect PRRSV RNA by rRT-PCR compared to bleeding 100% of piglets (Almeida et al., 2018). Furthermore, he demonstrated that 10 family fluids offered a 98% probability to detect the virus at 2% or higher prevalence in due-to-wean piglets.

In summary, new, population-based monitoring schemes for infectious disease monitoring and surveillance systems have been described (PF and FOF), and are being increasingly adopted in the field due to the increased practicability, and great sensitivity and specificity compared to conventional (pig bleeding) methods. However, there is still limited information on the dynamics of PRRSV detection over time by these methods in herds undergoing virus control and elimination (i.e. intent to produce PRRSV-negative piglets at weaning). There is a need to understand what is the minimum combination of PF, FOF, and/or serum sampling over time to provide a great confidence of lack of virus circulation in suckling pigs.

The purpose of this research was to characterize the dynamics of PRRSV detection over time in different age groups in breeding herds attempting to produce PRRSV-negative piglets at weaning, and generate field-based information to develop surveillance and monitoring systems for reliable PRRSV detection in breeding herds undergoing virus elimination.

Objectives:

The overall objective of this project was to describe the dynamics of PRRSV detection by rRT-PCR in suckling piglets over time in herds undergoing PRRSV elimination. This was accomplished by sampling pigs at 3-5 days of age using PF, and re-sampling the same group of pigs prior to weaning using FOF.

Intensively sampling pigs from the farrowing unit over time in herds undergoing PRRSV elimination using PF, FOF, and due-to-wean piglet serum generates solid field data to allow refining guidelines for PRRSV surveillance using a combination of sample types that maximizes the probability of virus detection at high, moderate, and low prevalence while optimizing diagnostic costs.

Materials & Methods:

Study design and sampling procedure: This was a field study conducted in 7 breeding herds undergoing PRRSV elimination with the main objective of describing the dynamics of PRRSV detection by rRT-PCR in the same cohorts using different sample types (PF at 3-5 days of age and FOF from due-to-wean piglets). PF consists of serosanguineous liquid derived from the process of castrating and tail docking piglets. FOF is the liquid obtained from cotton ropes exposed to lactating sow and respective due-to-wean piglets. PF was collected weekly from all rooms being processed each week during the study period, and up to 20 FOF samples were collected from a convenient selected population within each farm.

Diagnostic testing: All PF and FOF samples were tested individually (no pooling) by rRT-PCR to detect PRRSV RNA at the Iowa State University Veterinary Diagnostic Laboratory.

Statistical analysis: Descriptive statistics and agreement between sample types was described. Results are shown using plots describing test results of each herd over time (weeks), space (rooms) and age group (PF for 3-5 days old pigs, and FOF for due-to-wean litters).

Results:

The results section was organized in 2 sections: farm-by-farm results, and general observations with aggregated results. Results are also shown in a visual format with 1 figure per study farm, demonstrating test results over time (weeks) and rooms, by sampling type (PF and FOF).

Results by farm:

In the original proposal it was planned to collect 1 PF per week for 3 farms. We were able to recruit more farms, and most participants agreed to match funds to test PF more frequently than once/week, collecting PF per room, for most rooms throughout the study. This leveraged the funds provided by NPB.

Farm A (figure 1) was followed for 13 weeks using PF. Also, FOF samples were collected in four of those weeks. Two of the weeks that had results for both PF and FOF weeks were positive with

PF, but considered negative with FOF. However, rooms that tested positive with PF in those weeks were not tested with FOF.

Farm B (figure 1) was followed for 19 weeks with PF and FOF were also collected in 8 weeks. In the 8 weeks that had results for both PF and FOF PF detected 1 week as positive, while FOF detected the same week as negative (same room tested positive in PF and negative with FOF). In the same farm 3 weeks tested positive by FOF and negative with PF.

Farm C (figure 2) was followed for 19 weeks, for which we had 18 weeks of PF testing and 7 weeks of FOF testing. From those, two weeks were classified as positive with PF. Within those, week 38 had two rooms testing positive with PF, and one room testing positive with FOF. In week 39 two rooms tested negative with PF, but one room tested positive with FOF.

Farm D (figure 3) was followed for 17 weeks, for which all weeks were tested with PF and 7 weeks were tested with FOF. For the 7 weeks with PF and FOF results, 6 weeks had agreement in the results. However, week 34 had 4 rooms testing negative with PF, and the only room sampled with FOF tested positive.

Farm E (figure 4) was followed for 29 weeks for which all weeks were evaluated with PF, and 17 weeks were collected with FOF. Of those 17 weeks, 9 weeks had agreement in the results between PF and FOF (5 positive weeks and 4 negative weeks). Seven weeks were positive in PF, but negative in FOF. However, in 2 of those the room that was positive with PF was not tested with FOF and in one instance a room tested with FOF had unknown status with PF. One week tested negative with PF but positive with FOF.

Farm F (figure 5) was followed for 18 weeks for which all weeks were evaluated with PF and 15 weeks with FOF. All samples tested negative at all times.

Farm G (figure 5) was followed for 16 weeks in 8 batches (batch-farrowing system) with PF and FOF. One week tested positive in both PF and FOF. One week tested positive in PF but negative in FOF.

General observations with aggregated results:

Weekly intermittent results in PF defined as a week testing positive followed by a negative week, and subsequent positive result happened twice in farm A, twice in farm B, twice in farm C, four times in farm D, four times in farm E, and once in farm G. Intermittent results with PF within a week defined as rooms tested within the same week with different PRRSV status defined by PCR testing happened twice in farm A, once in farm B, once in farm C, twice in farm D, 15 times in farm E, and once in farm G.

Similarly, weekly intermittent results in FOF defined as a week testing positive followed by a negative week, and subsequent positive results happened twice in farm B, once in farm D, and four times in farm E. Intermittent results with FOF within a week defined as rooms tested within the same week with different PRRSV status defined by PCR testing happened three times in farm B, three times in farm C, three times in farm D, twice in farm E, and once in farm G.

Farm G had three consecutive batches (6 weeks' time) of PCR-negative results in PF during the monitoring period. During the monitoring period, 4 batches tested negative with FOF (10 weeks' time) before a room tested positive again with FOF. The longest period of consecutive negative results with PF in farm E was of 4 weeks, while FOF yielded a period of 11 weeks of negative results in which three weeks were not tested. Farm D had 4 consecutive weeks of negative results in PF during the monitoring period with subsequent positive results. There were 2 consecutive week negative results with FOF which also tested negative by PF. Farm C had 9 weeks of interval between positive weeks (with 8 negative weeks with PF and 1 week not tested), the last 3 weeks during that period also tested negative with FOF. Farm B tested 11 weeks

negative with PF with 2 of those weeks testing positive in FOF. The farm went on to have another 3 weeks of consecutive negative results with PF with one of those weeks testing positive with FOF. Farm A had 3 periods of 3 consecutive negative testing with PF during the monitoring period.

The overall agreement between PF and subsequent FOF results was 73%. The frequency in which FOF detected a herd-week as positive and PF tested negative that same week was 9.5%. On the other hand, in 17.5% of the time PF tested positive for a specific herd-week while FOF failed to detect the same week as positive (Table 1). However, not in all instances the same rooms testing positive with PF were tested with FOF. When only taking in consideration the results where the same rooms were tested by both PF and FOF, both techniques yielded the same classification for a given herd-week in 80.7% of the time. In those situations (matched PF-FOF results by room over time), PF classified a week as positive and FOF as negative in 8.8% of the occasions and FOF classified as week as positive in PF as negative in 10.5% of the time (Table 2).

Table 1. Overall agreement between PF and FOF on the classification of matching weeks tested by PCR for PRRSv RNA detection including all matching weeks (all herds combined).

		FOF		Overall agreement
		POSITIVE	NEGATIVE	
PF	POSITIVE	12 (19.0%)	11 (17.5%)	
	NEGATIVE	6 (9.5%)	34 (54.0%)	
	TOTAL	18 (28.6%)	45 (71.4%)	73.0%

Table 2. Overall agreement between PF and FOF on the classification of matching rooms tested by PCR for PRRSv RNA detection (all herds combined).

		FOF		Overall agreement
		POSITIVE	NEGATIVE	
PF	POSITIVE	12 (21.1%)	5 (8.8%)	
	NEGATIVE	6 (10.5%)	34 (59.6%)	
	TOTAL	18 (31.6%)	39 (68.4%)	80.7%

Plots for each farm with test results by week, room, and sample type (PF or FOF):

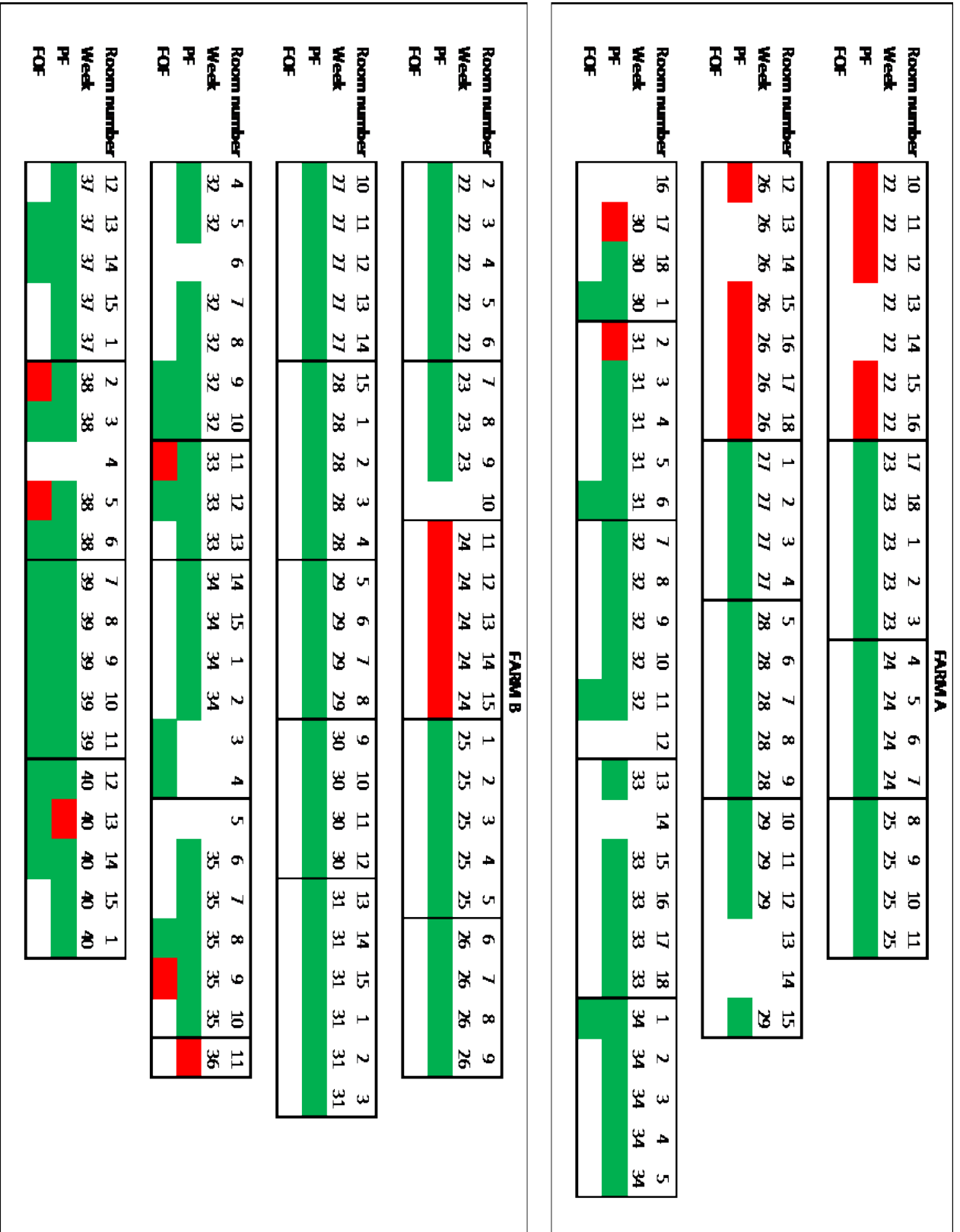


Figure 1. Results of PCR testing in processing fluids and family oral fluids over time (weeks), by room

Green PCR negative Yellow PCR suspect range Red PCR positive

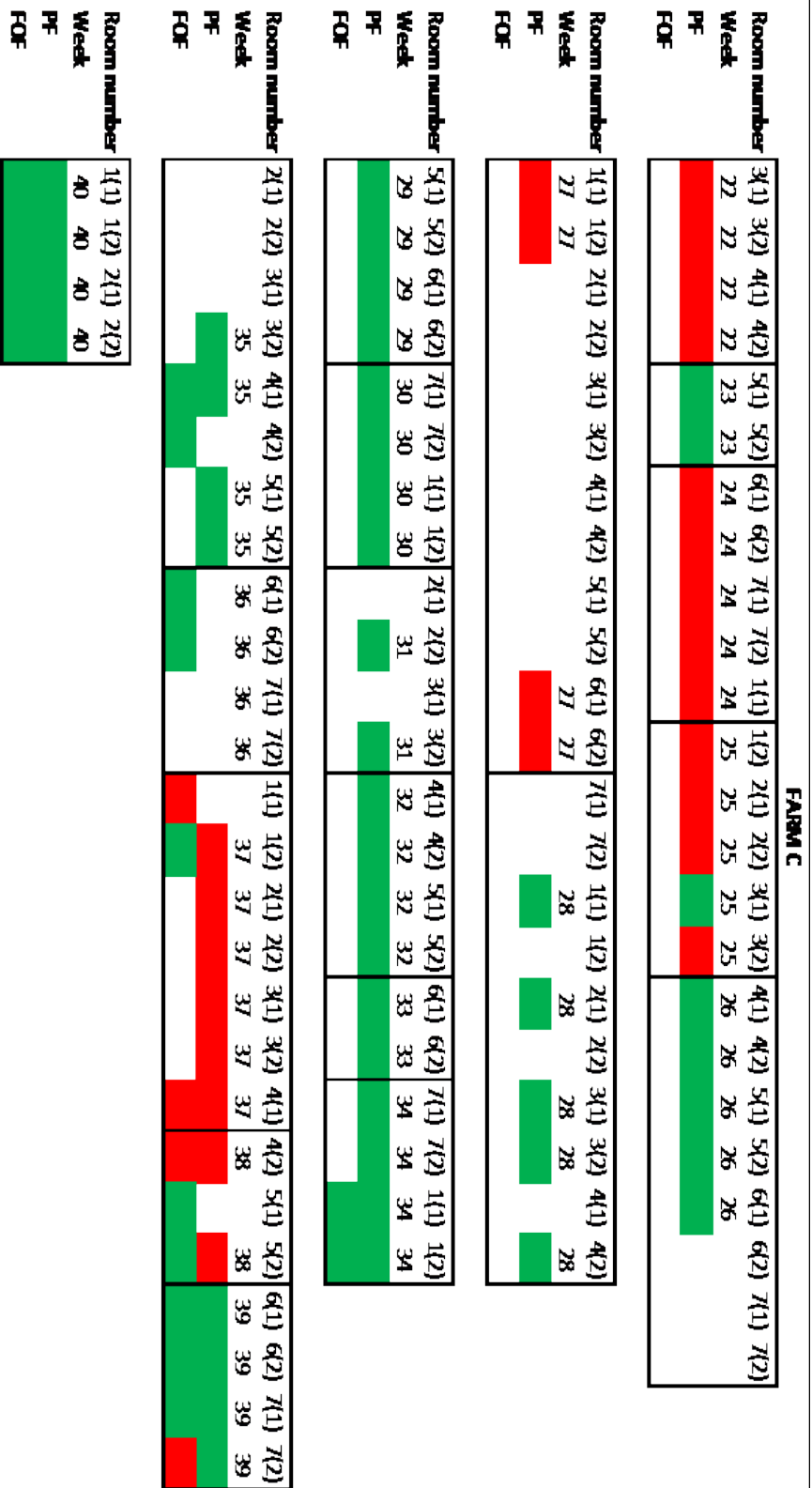


Figure 2. Results of PCR testing in processing fluids and family oral fluids over time (weeks), by room in farm C

PCR negative PCR suspect range PCR positive

FARM D

Room number	13	14	15	16	17	18	19	20	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Week	23	23	23	23	23	23	23	23	24	24	24	24	24	24	24	25	25	25	25	25	25	25
PF	Green																					
FOF	Green										Red											

Room number	15	16	17	18	19	20	21	21	21	27	27	27	28	28	28	29	29	29	29	29	29	30	30	30	30
Week	26	26	26	26	27	27	27	27	27	27	27	27	28	28	28	29	29	29	29	29	29	30	30	30	30
PF	Red																								
FOF	Red										Green														

Room number	19	20	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	1	2	3	4	1	2
Week	31	31	31	31	31	31	32	32					32	32	32	32	33	33	33	33	33	33	33	33	34	34	34	34
PF	Green																											
FOF	Green										Green										Red							

Room number	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	1	2	3	4	5	6	
Week	35	35	35	35						36	36		37	37	37	38		38	38	38	39	39	39	39	
PF	Green																								
FOF	Green										Red										Green			Red	

Figure 3. Results of PCR testing in processing fluids and family oral fluids over time (week), by room in farm D

Green PCR negative

Yellow PCR suspect range

Red PCR positive

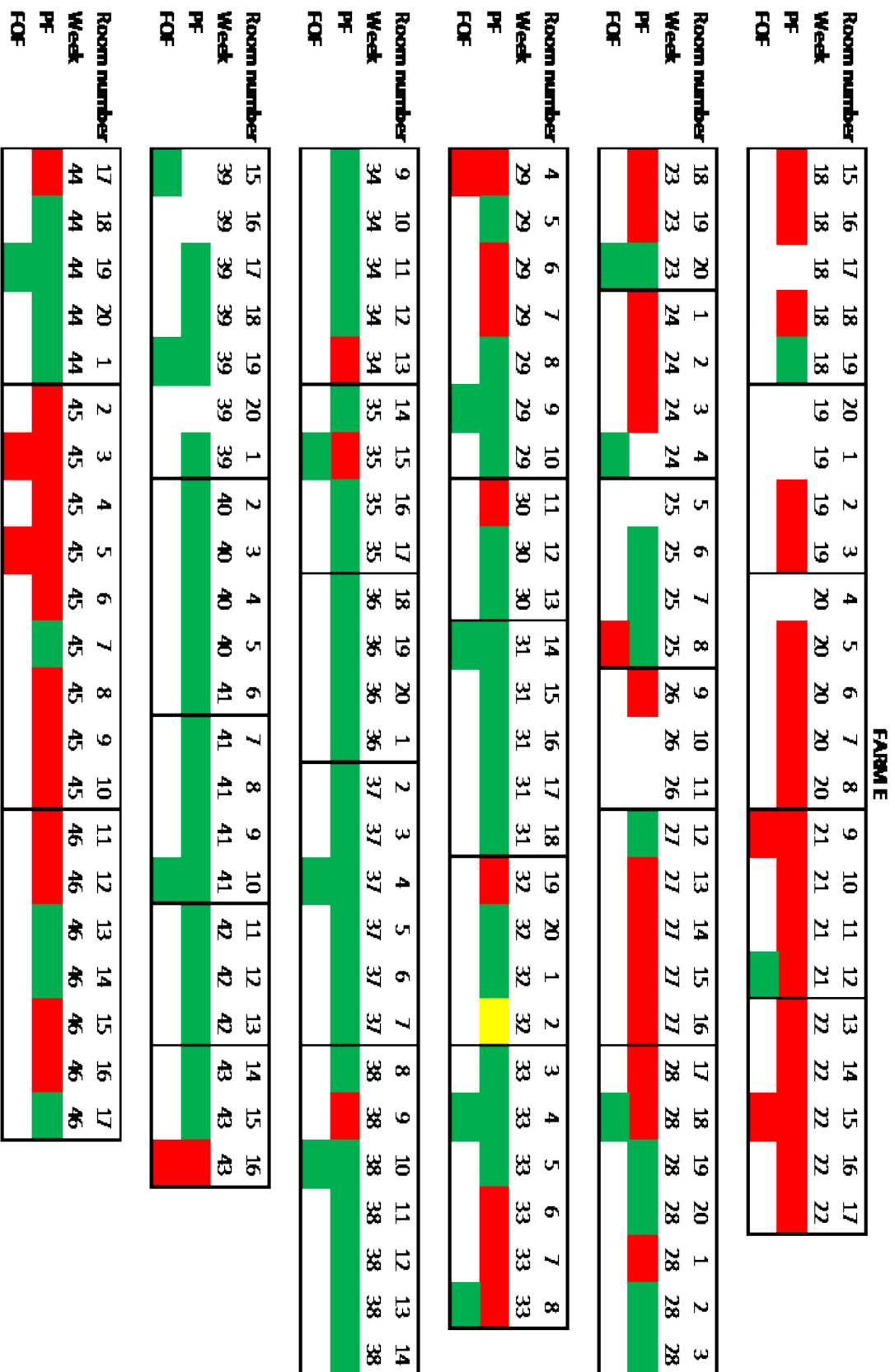


Figure 4. Results of PCR testing in processing fluids and family oral fluids over time (weeks), by room in farm E

Green PCR negative Yellow PCR suspect range Red PCR positive

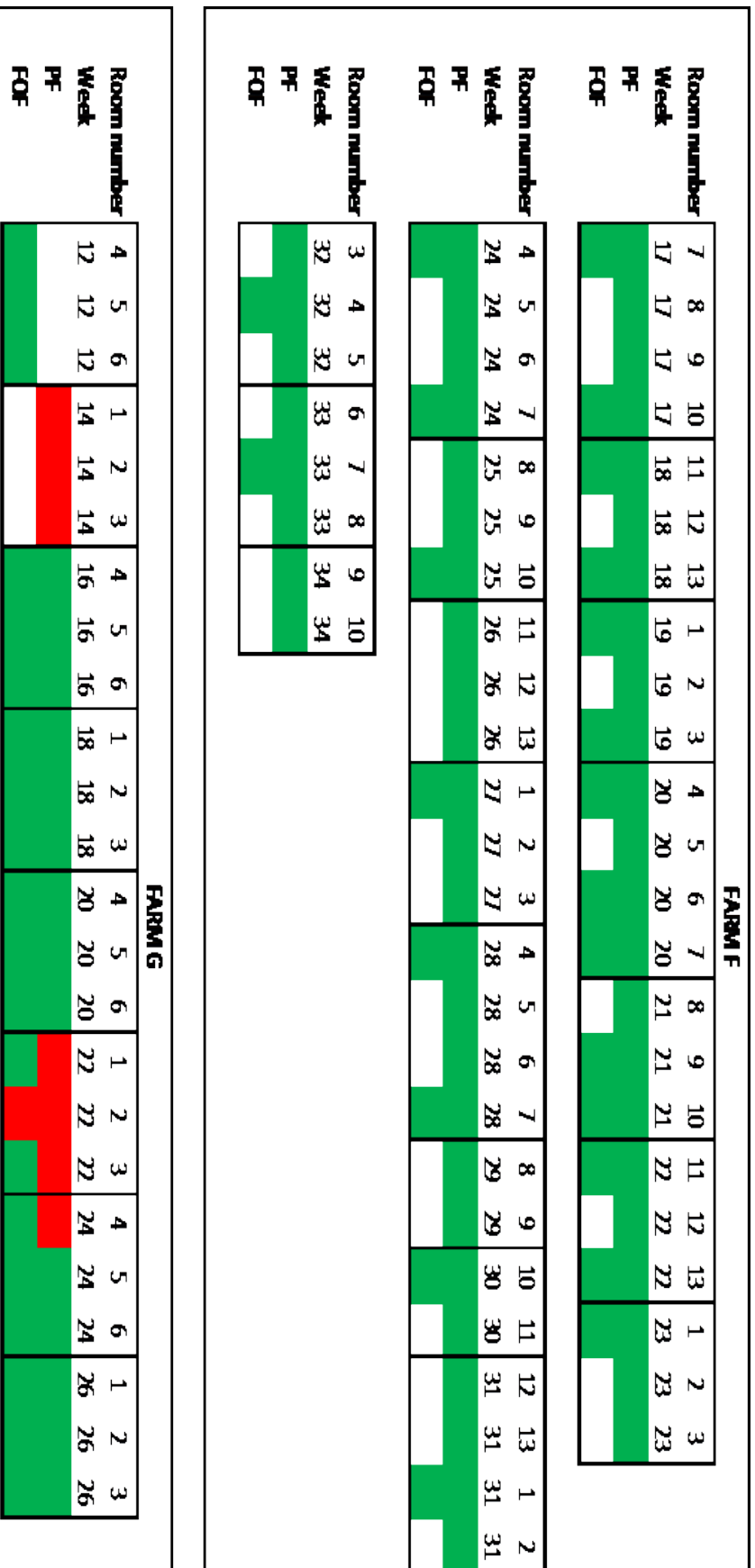


Figure 5. Results of PCR testing in processing fluids and family oral fluids over time (weeks), by room in farm F and G

Green PCR negative

Yellow PCR suspect range

Red PCR positive

Discussion:

This study provided new information on the dynamics of PRRSv RNA detection by rt-RT-PCR in processing fluids (PF) and family oral fluids (FOF) over time (weeks) and space (rooms).

The farms that had at least one positive sample (PF or FOF) during the study period did not achieve stability during the study period. Although one farm achieved 11 weeks of consecutive negative results with PF, testing piglets at weaning demonstrated the detection of PRRSV twice during that same period which demonstrates the importance of testing the due-to-wean piglet population for a higher confidence in the results of the monitoring program.

One of the main findings of this study was the nature of the PRRSv detection within and between weeks and rooms of a given herd. Among all farms there was intermittent weekly detection of PRRSv using PF in 15 occasions and using FOF in 7 occasions. Within the same week, intermittent results were observed 22 times with PF and 12 times with FOF between rooms. This clearly demonstrates 2 crucial factors that need to be incorporated in PRRSV monitoring in farms undergoing virus elimination: continuously monitoring on a weekly basis, and sampling as many rooms as possible in an attempt to minimize misclassification of farm based on the test results of a single room.

When considering mismatched results between PF and FOF hypotheses that could explain a positive result in PF followed by a negative result with FOF include 'not testing a room that was positive in PF with FOF'; 'low PRRSv prevalence among litters'; or 'mortality of piglets that were potentially viremic before they could be sampled with FOF'. One hypothesis that could explain a negative result in PF and subsequent positive FOF result with the same cohort could be a 'very low prevalence at the moment of processing', increase in the number of positive piglets in the cohort from time of processing to the weaning time due to transmission of the virus within the same cohort or 'lateral introduction of the virus by failure of internal biosecurity practices'.

These results provide insights for the design of improved diagnostic monitoring programs for breeding herds attempting PRRSv elimination by suggesting a combination of PF and FOF for an increased chance to detect the virus in the suckling pig population. Also provides evidence of the intermittent nature of detection in different weeks and by room within the same week with PF and FOF. This also provides evidence that a period of more than 11 weeks of consecutive negative results with PF and FOF is necessary to establish a herd as stable for PRRSv.

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