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Effect of litter aggregation and pooling on detection of porcine reproductive and respiratory virus in piglet processing fluids

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Keypoints:

- Detection of PRRSV in processing fluids where one positive animal is mixed with large groups of negative animals is dependent on the level of viremia together with the sex of the positive pig.
- Theoretically, low Ct values could be pooled/diluted more than 1,000 times and PRRSV could still be detected.
- Processing fluids aggregation and pooling should be adapted according to the different prevalence scenarios and health goals (e.g eradication vs control).

Background

The use of processing fluids (PF) to monitor PRRSV in breeding herds has increased in the last two years. The processing fluids (e.g. tails and testicles) is a great cost-effective method that allows us to sample large numbers of animals without losing much of the individual sensitivity. In a previous study we evaluated the sensitivity of processing fluid PCR results at the litter level to detect at least one positive PCR piglet in a litter. Results from that study showed a sensitivity of 89% (95%CI: 66% to 97%)[1]. One of the interesting findings from that study was that we were able to detect PRRSV in seven out of eight litters that had at least one positive piglet. Sensitivity of the PF was influenced by number of positive piglets in the sample and viral quantity in the serum sample. This study lead us to the next study aiming at understanding the effect of aggregating litter processing fluids on PRRSV detection.

Objective

Current practices in the field involve aggregating more than one litter and pooling PF from different days or rooms. In this study we assessed the effect of both practices in the detection of PRRSV in PF.

Methods and Results

Aggregation effect: Briefly, the tail from a conveniently selected positive female and tails and testicles from 2 conveniently selected positive males were placed in separate sterile plastic bags containing processing tissues from 10 PRRSV-negative litters (~120 piglets) obtained from a PRRSV-free herd. These 3 bags were stored at 4°C for 24 h after mixing the tissues. After this time, 4 mL of PF were extracted for RT-PCR testing, and then the processing tissues from 10 additional PRRSV-negative litters were added to each bag. The process was repeated until each bag contained processing tissues from 50 litters. Results of this study can be seen in Table 1.

Pooling effect: eight PRRSV RT-PCR-positive PF samples with Ct values between 20 and 35 were diluted 10, 20, 30, 40 and 50 times using PRRSV RT-PCR-negative PF samples. Sample dilutions were tested by RT-PCR at the UMN VDL and a linear model was built using the model's worst-case scenario slope to predict the number of dilutions possible before crossing the thresholds of suspect (35) and negative (40) Ct values. Results are summarized in Table 2.

Conclusion

The results of this study show that we can detect PRRSV after aggregating up to 50 litters, or above 1,000 dilutions when pooling. PRRSV detection with both methodologies is dependent on the level of positivity and the sex of the positive piglets since sensitivity is lower when a female pig was used as the source of virus in the aggregate sample. However, results of this study should be taken with caution and adapted to different prevalence scenarios and goals (eradication vs control). A more comprehensive study on the different strategies (number of litters to aggregate or number of PF to pool) that veterinarians and practitioners are currently using in the field and the time to declare a farm negative are needed.

Currently, our group has an ongoing study to understand and analyze current PF practices to evaluate the number of negative PF to declare a farm stable. If you are interested in participating in this study contact Dr. Juan Sanhueza (jsanhuez@umn.edu).

If you are interested in reading the full article go to the following link or contact MSHMP.

https://journals.sagepub.com/doi/full/10.1177/1040638719852999

[1]https://www.sciencedirect.com/science/article/pii/S0378113518308344?via%3Dihub

Piglet sex/Ct value*	Litters aggregated				
	10	20	30	40	50
Male					
22.1	Not tested	22.4	22.8	23.5	24.2
33.6	34.4	36.5	34.8	36.0	Negative
Female					
34.3	Negative	Negative	Negative	Negative	Negative

* Result of the blood swab collected from the tail and scrotum incision during processing.

Table 1. Summary of porcine reproductive and respiratory syndrome virus processing fluid RT-PCR cycle threshold results following tissue aggregation of 1 positive piglet with 10, 20, 30, 40, or 50 PRRSV-negative litters.

Initial Ct	Dilution							Maximum dilution to cross the threshold (35 Ct/40 Ct)
	10	20	30	40	50	100	1,000	
35	*38.7 (0%/95.9%)*	*39.8 (0%/60.5%)*	**40.5 (0%/25.3%)**	**40.9 (0%/11.5%)**	**41.3 (0%/4%)**	**42.4 (0%/0%)**	**46.1 (0%/0%)**	1/22
30	33.7 (95.2%/100%)	34.8 (59.5%/100%)	*35.5 (26.5%/100%)*	*35.9 (10.7%/100%)*	*36.3 (4.3%/99.9%)*	*37.4 (0.1%/99.9%)*	**41.1 (0%/6.9%)**	22/500
25	28.7 (100%/100%)	29.8 (100%/100%)	30.5 (100%/100%)	30.9 (100%/100%)	31.3 (99.9%/100%)	32.4 (99.9%/100%)	36.1 (6.9%/99.9%)	500/11,200
20	23.7 (100%/100%)	24.8 (100%/100%)	25.5 (100%/100%)	25.9 (100%/100%)	26.3 (100%/100%)	27.4 (100%/100%)	31.1 (99.9%/100%)	11,200/251,000

Values in parentheses are the % of times that the Ct values would be expected to be below the suspect (>35 Ct) and negative (≥ 40 Ct) thresholds using a normal distribution. Areas in which the predicted Ct average was over the suspect and negative thresholds are shaded in italic and boldface, respectively.

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