RESEARCH REPORT
SWINE HEALTH

TITLE: Development of on-farm PRRSV surveillance guidelines for the modern pork industry – (NPB #13-157)

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Industry Summary

Oral fluids (OF) are a convenient surveillance sample because they (1) are easily collected by a single person; (2) can be collected frequently without stress to pigs or people; and (3) provide a higher probability of analyte detection with fewer samples than serum (Olsen et al., 2013). The goal of this research was to develop guidelines for PRRSV surveillance.

In 3 commercial wean-to-finish barns on one finishing site, OFs were collected weekly from every occupied pen (108 pens; ~25 pigs per pen) for 8 weeks (total of 972 OF samples. These samples were completely randomized and then tested for PRRSV by RT-PCR.

The probability of PRRSV detection by RT-PCR was derived as a function of sample size (Table 1) and sample allocation (random vs spatial). Notably, systematic spatial sampling was shown to be as good, or better, than random sampling for the detection of PRRSV infection. That is, regardless of the number of samples collected, spacing of samples equidistantly over the length of the barn provided for the highest likelihood of detection.

Analysis also showed that PRRSV exhibited spatial autocorrelation at the barn level (Moran's *I* analysis). This result provided further support to the conclusion that systematic spatial sampling was a valid approach, i.e., Aune-Lundberg and Strand (2014) state, "*systematic sampling is more precise than simple random sampling when spatial autocorrelation is present and the sampling effort is equal*."

Producers and swine veterinarians should design a sampling plan that will meet their goals for surveillance. For PRRSV detection, the following should be taken into account: (1) all buildings should be sampled because the pattern of infection differs among buildings; (2) sample size should be selected based on the budget allocated for surveillance and the detection target. For example, as shown in Table 1, 6 samples collected using a systematic spatial plan have an 85% probability of detecting PRRSV if 25% of the pens are positive.

Keywords: PRRSV, surveillance, monitoring, oral fluids, RT-PCR

Scientific abstract

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Introduction

Producers need to know the PRRSV status of their herds to make good decisions, but surveillance cannot be burdensome or costly. As discussed by Klaucke et al. (1998), a successful surveillance system will be simple, flexible, accurate, timely, and acceptable to the users.

"Representative sampling", i.e., testing a subset of a population, was the first development towards more efficient surveillance. Proposed in 1895 (Kruskal and Mosteller, 1980), representative sampling was not applied to swine surveillance until the 1970's when statistical sampling in the PRV eradication program replaced the whole-herd testing used in the "hog cholera" eradication program. Thirty serum samples became the standard for herd surveillance, thereafter (Anderson et al., 2008). Notably, while adequate for the small farms of the 1980's, surveillance based on the "30 serum samples" formula is out of step with the size, structure, and dynamic nature of today's industry.

In the last decade, oral fluid samples have become a viable alternative to serum for surveillance. Beginning with NPB-funded research (*#05-146 - An improved method for PRRSV surveillance and monitoring*), a series of research and field studies showed the utility of oral fluids for the detection of PRRSV and other pathogens using either PCR- or antibody-based approaches (Kittawornrat et al., 2010, 2013; Prickett et al., 2008a,b; Ramirez et al., 2012). Oral fluid specimens offer specific advantages for surveillance: (1) they are easily collected (2) they can be collected as often as needed without stress to pigs or people and (3) they provide

a higher probability of PRRSV detection with fewer samples than serum (Olsen et al., 2013). The problem at this point in time is that we do not have statistically-based guidance regarding imp

Objectives.

The objective of this research was to derive statistically valid sampling on-farm guidelines for PRRSV surveillance based on oral fluids, i.e., sample size and sample allocation.

Materials and methods

In 3 wean-to-finish barns on one finishing site, OF samples were collected weekly from every occupied pen (108 pens; ~25 pigs per pen) for 8 weeks following placement. A total of 972 OF samples were collected. OF samples were completely randomized and then tested for PRRSV by RT-PCR.

Longitudinal PRRSV RT-PCR binary results over time were analyzed for: (1) the presence of PRRSV spatial autocorrelation using threshold distance as the spatial weight matrix. Moran's *I* was used to test for global spatial autocorrelation, i.e., for clustering of PRRSV virus within the barns. LISA (Local Indicators of Spatial Association) were used to test for local spatial autocorrelation, i.e., identify specific PRRSV clusters within the barns. **(2)** Results were also analyzed using a piecewise exponential survival model for interval censored time to event data with misclassification. The unobserved true disease status was modelled through binary latent survival process which follows a piecewise exponential model. The hazard of disease onset for a certain pen in this survival model was a function of the other pens' disease statuses in the building and their distances to this pen, which takes into account the spatial transaction among pens. The diagnostic test outcomes were modeled conditional on the latent disease process using Bernoulli distribution parametrized through the sensitivity and specificity of the diagnostic test. Since the pens were sampled at pre-determined time points (weekly), the true disease onset time can be viewed as interval censored. The model parameters were estimated through a hierarchical Bayes approach utilizing non-informative priors. The model and the estimated parameters from real data analyses were then utilized to study the effect of sample number, location (spatial, random), and frequency on PRRSV detection through simulation studies.

Results.

Figure 1 shows the distribution of PRRSV RT-PCR-positive pens by barn and over time. Table 1 gives the probability of detecting PRRSV infection as a function of the number of oral fluid samples collected.

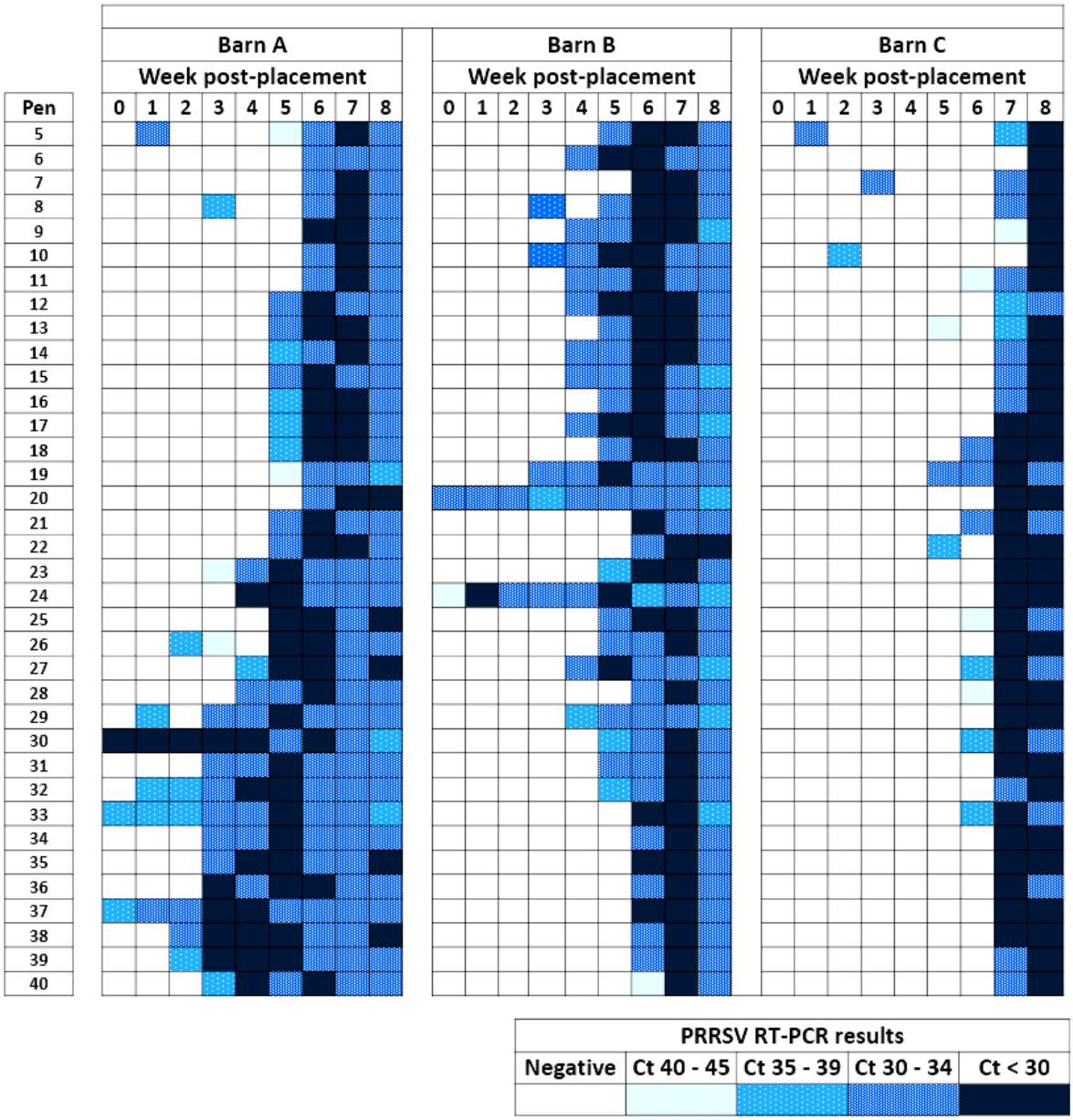

Figure 1. PRRSV RT-PCR results over time in 3 wean-to-finish barns on one site. Note that the progression of PRRSV differed among barns. For example, the infection moved slowly in Barn A until 4-5 weeks post-placement. In contrast, it spread extremely quickly after week 6 in Barn C.

Table 1. Probability of detecting PRRSV infection by number of pens with positive pigs (% positive) and number of pens sampled (one oral fluid sample per pen)*.

	Number of PRRSV-positive pens (% positive)									
SAMPLE SIZE	**1 (3)**	**2 (6)**	**3 (8)**	**4 (11)**	**5 (14)**	**6 (17)**	**9 (25)**	**18 (50)**	**27 (75)**	**36 (100)**
1	3	5	8	12	14	17	26	55	83	100
2	6	11	16	22	27	32	46	80	98	
3	8	16	23	30	38	45	62	92	100	
4	11	21	31	40	47	54	72	97		
5	13	26	36	46	55	63	80	99		
6	17	31	43	54	63	70	85	100		
9	25	45	60	72	80	86	96			
18	49	75	89	95	98	99	100			
27	75	94	99	100	100	100				
36	100	100	100							

Example with Table 1: If 25% of the pens are positive, 6 samples will have an 85% probability of detecting PRRSV.

*Assumptions: 1) systematic spatial sampling and 2) test diagnostic sensitivity = 100%, test diagnostic specificity = 100%

Discussion

The purpose of disease surveillance is to assure animal health and welfare, improve producer profitability, and protect a valuable national asset. With our reliance on international markets, the economic viability of U.S. swine producers is synonymous with the perceived health status of the U.S. national herd. The National Pork Board recognizes the need for surveillance and has set the goal of a "comprehensive and integrated swine health surveillance system" (Anon, 2010).

Although the focus was on PRRSV, the guidelines developed in this project are an important step toward designing oral fluid-based surveillance for a variety of pathogens, including transboundary and foreign animal diseases. The proposed approach differs significantly from historical surveillance concepts in that it is based on systematic spatial sampling of pen-based oral fluid specimens: (1) spatial sampling is compatible with the rapid turnover of swine populations and (2) oral fluid specimens are more sensitive than individual serum samples for the detection of either antibody or nucleic acid in populations (Olsen et al., 2013). Furthermore, approach incorporates the ideal surveillance attributes described by Klaucke et al. (1998): simple, flexible, accurate, timely, and acceptable to users.

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