Veterinary Microbiology 209 (2017) 20–29

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Sampling guidelines for oral fluid-based surveys of group-housed animals

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 5 February 2017
Accepted 9 February 2017

Keywords:
Oral fluid
Sampling
Surveillance
Sample size
PRRSV
Modeling
Probability of detection
Monitoring

ABSTRACT

Formulas and software for calculating sample size for surveys based on individual animal samples are readily available. However, sample size formulas are not available for oral fluids and other aggregate samples that are increasingly used in production settings. Therefore, the objective of this study was to develop sampling guidelines for oral fluid-based porcine reproductive and respiratory syndrome virus (PRRSV) surveys in commercial swine farms. Oral fluid samples were collected in 9 weekly samplings from all pens in 3 barns on one production site beginning shortly after placement of weaned pigs. Samples (n = 972) were tested by real-time reverse-transcription PCR (RT-rtPCR) and the binary results analyzed using a piecewise exponential survival model for interval-censored, time-to-event data with misclassification. Thereafter, simulation studies were used to study the barn-level probability of PRRSV detection as a function of sample size, sample allocation (simple random sampling vs fixed spatial sampling), assay diagnostic sensitivity and specificity, and pen-level prevalence. These studies provided estimates of the probability of detection by sample size and within-barn prevalence. Detection using fixed spatial sampling was as good as, or better than, simple random sampling. Sampling multiple barns on a site increased the probability of detection with the number of barns sampled. These results are relevant to PRRSV control or elimination projects at the herd, regional, or national levels, but the results are also broadly applicable to contagious pathogens of swine for which oral fluid tests of equivalent performance are available.

1. Introduction

As reviewed by Christensen (2001), various definitions of surveillance and monitoring appear in the literature, with the primary difference that surveillance implies that an action will be taken in the case of a positive result. However, as discussed in the FAO "Manual on Livestock Disease Surveillance and Information Systems" (Paskins, 1999), "surveillance" is often used interchangeably with "monitoring" (even by epidemiologists) and, in practice, the distinction between the two is often blurred. Paskins (1999) goes on to define surveillance as, "*All regular activities aimed at ascertaining the health status of a given population with the aim of early detection ....*" and monitoring as '*All activities aimed at detecting changes in the epidemiological parameters of a specified disease*'. Consistent with this approach, the assumption in this paper is that the purpose of surveillance is to detect infectious agents and the purpose of monitoring to detect changes in pathogens' trends in populations. Regardless of the purpose for which samples are collected, the sampling guidelines reported herein apply equally to both.

Beginning in the 20th century and continuing into the present, pig production moved from relatively small, extensive, labor-dependent enterprises into larger, intensive, technified production systems. In these farms, animals are segregated by age, production stage, and/or function – with little interaction between groups. Both breeding and growing pig populations turn over rapidly, but non-uniformly, as animals finish the production cycle and are replaced by others – often of differing infectious and/or immune status. Thus, the size and structure of contemporary production

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http://dx.doi.org/10.1016/j.vetmic.2017.02.004

systems leads to instability in herd immunity and promotes the circulation of agents. Schwabe (1982) recognized the impact of these changes on the expression of disease and prescribed continuous monitoring as a means of discovering the levels and patterns of pathogen circulation and evaluating the effectiveness of interventions.

In conjunction with these changes and particularly in North America and Europe, large numbers of young pigs are moved from breeding farms to finishing farms located in proximity to the areas where crops are produced. Thus, not exclusively, but primarily for this reason, 27,500,000 live animals entered the state of Iowa USA between December 1, 2014 and December 1, 2015 (NASS, 2016). While it is more cost-effective to bring the pigs to the feed (rather than the reverse), this management practice effectively connects distant farms and rapidly moves infectious agents between them. Ultimately, movement of large numbers of pigs compromises the ability of veterinary health authorities to control the spread of infectious diseases at the regional and national levels. This is of particular concern for transboundary and OIE-listed pathogens.

Cumulatively, these recent developments drive the need to collect infectious disease information more rapidly and efficiently. Historically, swine surveillance has been based on individual animal sampling, e.g., serum, nasal swabs, tonsil biopsies, etc., but aggregate specimens, such as oral fluids, offer specific advantages. In particular, oral fluid specimens can be collected by a single person, can be collected frequently (even daily) without stress to pigs or people, and can provide a higher probability of analyte detection with fewer samples than serum (Olsen et al., 2013). This approach provides for an inexpensive, practical, and welfare-friendly method to surveil pig populations. Detection of nucleic acids or antibodies in oral fluids have been reported for most swine pathogens, including *Actinobacillus pleuropneumoniae* (Loftager et al., 1993), African swine fever virus (Greig and Plowright, 1970; Giménez-Lirola et al., 2016), classical swine fever virus (Corthier and Aynaud, 1977), foot-and-mouth disease virus (Eblé et al., 2004; Senthilkumaran et al., 2016a; Vosloo et al., 2015), influenza A virus (Goodell et al., 2013; Panyasing et al., 2013), porcine circovirus type 2 (Prickett et al., 2011), porcine epidemic diarrhea virus (Bjustrom Kraft et al., 2016), porcine reproductive and respiratory syndrome virus (Kittawornrat et al., 2010, 2012, 2013; Prickett et al., 2008a, 2008b), swine vesicular disease virus (Senthilkumaran et al., 2016b), vesicular stomatitis virus (Stallknecht et al., 1999), and others.

The general need for a new surveillance approach reflects the requirement to adapt to the population structure and production practices in use on contemporary swine farms and the availability of new sampling/testing methods. The specific objective of the present study was to develop sampling guidelines for oral fluid-based porcine reproductive and respiratory syndrome virus (PRRSV) surveillance or monitoring in commercial swine farms. Estimates for probability of detection are needed to expedite on-farm data collection and aid in PRRSV control and/or eradication efforts.

2. Materials and methods

2.1. Experimental design

Oral fluid samples were collected in 9 weekly samplings from all occupied pens (~25 pigs per pen, 36 pens per barn) in 3 commercial wean-to-finish (WTF) barns on one production site in the Midwest USA. The Iowa State University Office of Responsible Research reviewed and approved the on-farm sampling procedures. After the final collection, the 972 oral fluid samples (36 pens x 3 barns x 9 samplings) were randomized and tested for PRRSV RNA by real-time reverse transcription polymerase chain reaction (RT-rtPCR). Longitudinal binary diagnostic test outcomes were analyzed using a piecewise exponential survival model for interval-censored, time-to-event data with misclassification. The model and the parameters estimated from analyses of field data were then used in simulations (10,000) to study the barn-level probability of PRRSV RNA detection in the context of sample size, sample allocation (fixed spatial vs simple random sampling), assay diagnostic sensitivity and specificity, and the number of positive pens. The effect of disease spread on probability of detection by time was evaluated using simulation studies for three scenarios; the observed spread of the infection (β_1, β_2), one-half the observed spread of the infection ($\beta_1/2$, $\beta_2/2$), and twice the observed spread of the infection ($2\beta_1$, $2\beta_2$).

2.2. Animals and animal care

The study was conducted on one swine farm with three curtain-sided, wean-to-finish barns (13.4 m × 61.0 m) sited parallel to each other and spaced 10 m apart. Barns used split-zone ventilation, with independent control of curtains and ridge ventilation by zone. Manure was collected in shallow pits beneath each barn and moved to an outdoor above-ground slurry storage tank via a scraper system. The site functioned on an all-in-all-out basis, with buildings cleaned and disinfected between groups. Animal veterinary care, housing, handling, and feeding were under the supervision of production system veterinarians.

Each barn contained 40 pens with 20 pens on either side of a central walkway. Pens (3 m × 6 m) were built with solid concrete walls and partial slats. At the time of the study, 36 pens in each barn were occupied, with ~25 pigs in each pen. Barns were filled with weaned pigs (~21 days of age) sourced from the same PRRSV-endemic breeding herd over the course of approximately one week. Commercial modified-live PRRS vaccines were administered to replacement gilts in the breeding herd, but PRRS vaccine was not administered to sows or pigs.

2.3. Sample collection

Oral fluid samples were collected weekly from each of the 36 occupied pens in each of the 3 barns, i.e., 108 samples per week, using a procedure described elsewhere (Prickett et al., 2008a, 2008b). In brief, oral fluid samples were collected by hanging one 100% cotton rope in each pen, with the end of the rope hanging at the height of the pigs' shoulder. One day before the first sample was collected, pigs were “trained” by providing access to ropes for 60 min (White et al., 2014). For routine sampling, ropes were hung for 20–30 min. Thereafter, the wet portion of the rope was inserted into a one gallon plastic bag and severed from the remainder of the rope. Oral fluid was extracted by passing the rope, still within the bag, through a chamois wringer. Samples were decanted into 50 mL centrifuge tubes and placed on crushed ice for transport to the laboratory. At the laboratory, samples were aliquoted into cryovials (4 mL) and stored at −20 °C. Prior to testing, samples were placed in random order to control for systematic bias. Sampling began one week after pigs were placed in the facility and continued for 8 weeks thereafter (total of 9 samplings).

2.4. PRRSV RT-rtPCR

All samples were tested for the presence of PRRSV RNA at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) using standard protocols. Extraction of the oral fluids was performed using the MagMAX™ viral RNA isolation kit (Life Technologies, Carlsbad, CA, USA) and a Kingfisher 96 magnetic particle processor (Thermo-Fisher Scientific, Waltham, MA, USA) using a high-volume modified lysis (HVML) procedure. A modified

lysis/binding solution was prepared with 120 μL lysis/binding solution, 2 μL carrier RNA, 120 μL isopropanol and 2 μL Xeno™ RNA template at 10,000 copies/μL. At the lysis step, 240 μL of the prepared lysis/binding solution was added to 20 μL of magnetic bead mix prior to extraction and elution into 90 μL buffer. An additional modification for the HVML procedure was an increase in volume of wash I and II solutions, i.e., the procedure used 300 μL in wash I and 450 μL in wash II. The extraction was performed using Kingfisher AM1836_DW_HV_v3, provided by Thermo Fisher Scientific.

Samples were assayed using a commercial PRRSV real-time rtPCR kit (EZ-PRRSV MPX 4.0 assay, Tetracore©, Rockville, MD, USA). For each run, one positive control for PRRSV Types 1 and 2 and a negative amplification control were included. For each control well, 17.25 μL of EZ-PRRSV MPX 4.0 Reagent was added. The EZ-PRRSV MPX 4.0 Reagent includes buffer, primer and probes, 0.75 μL Enzyme Blend, 0.25 μL IC and 7 μL of positive control (Type I or 2 IVT) or negative control (1x TE). Specifically for oral fluid samples, each well contained 17.25 μL of the EZ-PRRS MPX 4.0 Reagent, which included buffer, primer, probes, 0.75 μL Enzyme Blend and 7 μL of the oral fluid extract. Plates were loaded onto the thermal cycler (7500 Fast Real-Time PCR System, Applied Biosystems©, Foster City, CA, USA) and the following cycling conditions were used: one cycle at 48 °C for 15 min, one cycle at 95 °C for 2 min, 45 cycles of: 95 °C for 5 s, and 60 °C for 40 s. Samples with Ct values <45 for Type 2 PRRSV were considered positive.

2.5. Statistical analysis

Longitudinal binary diagnostic test outcomes, $u_{ij} = (u_{ij1}, \ldots u_{ijt})$, for pen j in barn i and sampling time t were analyzed using a piecewise exponential survival model for interval-censored time-to-event data with misclassification (Sun, 2017). The corresponding unobserved true infection status, $y_{ij} = (y_{ij1}, \ldots y_{ijt})$, was modeled through a binary latent survival process that followed a piecewise exponential model. The hazard of the onset of infection for pen j in the survival model, λ_{ijt}, was modeled as a function of the infection status of the other pens j' in the building and the distance ($d_{jj'}$) between pens j and j' within the same barn:

$$\lambda_{ijt} = \exp\left\{-\left[\beta_0 + \sum_{j' \neq j}\left(\beta_1 + \beta_2 \frac{1}{d_{jj'}}\right) y_{ij', t-1}\right]\right\}. \quad (1)$$

Here β_0 is the baseline negative log-hazard and β_1 and β_2 are parameters quantifying the spread of the infection. Specifically, β_1 represents the change in log-hazard for each additional positive pen in the same barn regardless of distance and β_2 represents the change in log-hazard for each additional positive pen in the same barn per $1/d_{jj'}$.

Diagnostic test outcomes were modeled conditional on the latent disease process using Bernoulli distribution parametrized through the assay's diagnostic sensitivity (se) and specificity (sp):

$$u_{ijt} \mid (y_{ijt} = 1) \sim \text{Bernoulli(se)},\ u_{ijt} \mid (y_{ijt} = 0) \sim \text{Bernoulli(1-sp)}. \quad (2)$$

These test outcomes are correlated over space and time as a result of the model structure.

Since the pens were sampled at pre-determined time points, t (weekly), the true infection onset time can be viewed as interval-censored. The model parameters β_0, β_1, and β_2 were estimated through a hierarchical Bayes approach utilizing non-informative priors. The model and the parameters estimated from analyses of field data were then used in simulation studies to study the effect of sample size, sample allocation (simple random sampling or fixed spatial sampling), and sampling frequency on the probability of detecting PRRSV infection while controlling for assay diagnostic sensitivity and specificity, prevalence (proportion of positive pens), and spread of the virus. For any selected sample size, pen samples were either randomly selected using software *R* 3.2.2 (R Development Core Team, 2015) or selected using a fixed spatial sampling approach. Fixed spatial sampling was based on selecting pens equidistant to each other and on alternate sides of the center alleyway over the length of the barn.

2.5.1. Probability of detection in a single barn (single sampling)

The probability of detection in a single barn at a single sampling was evaluated for a range of relevant criteria, i.e., diagnostic sensitivities and specificities, sample sizes (1–36), sample allocation (simple random sampling vs. fixed spatial sampling), and prevalence (0–36 positive pens). Simulations were carried out in *R* 3.2.2. In each simulation study, the true infection status of the 36 pens in each of the 3 barns was simulated over time using the estimated model parameters (β_0, β_1, β_2). For each set of sampling criteria, the probability of detection was calculated as the proportion of simulations (out of 10,000 runs) with ≥1 positive pens among the total pens sampled.

Infection status and sample test outcomes were generated using simulation studies over the sampling period of 8 weeks, $t = 0, 1, \ldots, 8$. For each pen j in barn i, the true infection status, y_{ij0}, at the initial sampling point was generated from the Bernoulli distribution with probability p_0., the initial prevalence at week 0. If the result was $y_{ij0} = 1$, the pen was classified positive at sampling point 0 and all subsequent sampling periods. If the result was $y_{ij0} = 0$, the time to positive pen status t_{ij1} was simulated from an exponential distribution with parameter λ_{ij1} defined as in (1), where $y_{ij'0}$ was the true infection status for pen j'at sampling time 0. If $t_{ij1} \leq 1$, then the true infection status for pen j at time 1 was $y_{ij1} = 1$, thus $y_{ij2} = \ldots = y_{ij8} = 1$. If $t_{ij1} > 1$, t_{ij2} was generated from an exponential distribution with parameter λ_{ij2}, as defined in (1), where$y_{ij'1}$ was the true infection status for pen j'at sampling time 1. If $t_{ij2} \leq 1$, then the true infection status for pen j at sampling time 2 was $y_{ij2} = 1$, thus $y_{ij3} = \ldots = y_{ij8} = 1$. If $t_{ij2} > 1$, t_{ij3} was generated from an exponential distribution with parameter λ_{ij3}, as defined in (1), where$y_{ij'2}$ was the true infection status for pen j'at sampling time 2. Similarly, the true infection status for each pen at each sampling point was generated through this procedure.

After simulation of infection status, diagnostic test outcomes were simulated with the number of pens sampled (1–36) allocated using either simple random sampling or fixed spatial sampling. For any predetermined level of diagnostic sensitivity or specificity, the test outcome, u_{ijt}, was generated conditionally on y_{ijt} from (2). At each prevalence level, the probability of detection was calculated as the proportion of simulations (out of 10,000 simulations) with ≥1 positive pen among the total pens sampled. The probability of detection was calculated for both simple random sampling and fixed spatial sampling and the results compared using McNemar's test for paired proportions.

2.5.2. Effect of the spread of infection on the probability of detection

As shown in Eq. (1), the spread of infection was controlled by β_1, β_2 such that larger values of β_1, β_2 resulted in faster spread among pens within a barn, while smaller values of β_1, β_2 produced slower spread. The effect of spread on the probability of detection by time in a single barn was explored by changing the values of these parameters in simulation studies. Fixed spatial sampling was used with sample sizes 2, 4, and 6 while allowing prevalence to change over time. For simplicity, diagnostic sensitivity and specificity were assumed to be 100%.

The effect of the spread of infection on the probability of detection was evaluated for three scenarios while keeping p_0, β_1 constant: the observed spread (β_1, β_2), one-half the observed

spread ($\beta_1/2$, $\beta_2/2$), and twice the observed spread of infection ($2\beta_1$, $2\beta_2$). Simulation studies were carried out and the true infection status at each sampling point was generated using the methods described above through the end of the sampling period (8 weeks). Test outcomes were generated conditional on the true infection status. At each sampling point, the probability of detection was calculated as the proportion of simulations (out of 10,000 simulations) with ≥1 positive pen among the total pens sampled.

2.5.3. Effect of sampling 2 or more barns on a site

The approach described above estimates the probability of detecting infection in one barn. Assuming independence among barns, the overall probability of detecting infection on one production site by sampling ≥2 barns can be calculated as:

$$P = (1 - (1 - p_1)(1 - p_2)(1 - p_3) \ \ldots \ (1 - p_k)). \quad (3)$$

In Eq. (3), p_i is the probability of detection in the ith ($i = 1,2, \ldots, k$) barn. When the k barns are similar in design and are sampled with same scheme, then all p_i can be assumed equal to a common p of detection and the formula simplifies to:

$$P = (1 - (1 - p)^k). \quad (4)$$

3. Results

Oral fluid samples were completely randomized prior to testing for PRRSV nucleic acid and then tested in batches of ~252 samples to optimize laboratory throughput. RNA extraction (Life Technologies) and RT-rtPCR (Tetracore, Inc.) were each performed using a single production lot. Samples were tested once, i.e., no retests were performed. A total of 425 samples tested positive (Ct ≤ 45) and 547 samples tested negative. The mean Ct among positives was 30.7 (95% confidence interval 30.4, 30.9). Table 1 provides a spatiotemporal perspective of the results. Descriptively, the 3 barns differed by the week at which they reached ≥4 PCR-positive pens (≥11% positivity): Barn A at week 1, Barn B at week 3, and Barn C at week 6. Likewise, barns differed in the time it took for PRRSV to spread from ≥4 positive pens to ≥32 (≥89%) positive pens: Barn A 4 weeks, Barn B 3 weeks, and Barn C 1 week.

Test results were used to estimate model parameters p_0, β_0, β_1, and β_2 through a hierarchical Bayes approach using non-informative priors with JAGS Version 4.0.0 (Plummer, 2015). Posterior means, standard errors, and 95% credible intervals are given in Table 2. The 95% credible intervals did not include 0, indicating that the parameters' estimates were statistically significant and that the constructed model effectively represented the spread of infection. The parameter estimates were then used in simulation studies, as described in Sections 2.5.1 and 2.5.2, to evaluate the effect of sample size, sample allocation (simple random sampling vs fixed spatial sampling), and time on the probability of detecting PRRSV infection in a single barn while controlling for assay diagnostic sensitivity and specificity, prevalence (proportion of positive pens), and spread of infection.

Simple random sampling and fixed spatial sampling were compared in terms of the probability of detecting ≥1 positive samples over a range of sample sizes and number of positive pens in a single barn. For simplicity, the data presented in Fig. 1 assume that diagnostic sensitivity and diagnostic specificity are both 100%. The results for each set of parameters were based on 10,000 simulations, i.e., the standard errors for each estimate should be smaller than 0.005. Comparisons of the results showed that the probability of detection using fixed spatial sampling was equal to, or greater than, the probability of detection using simple random sampling (McNemar's test, $p < 0.05$). Therefore, the remainder of the analyses reported herein were based on fixed spatial sampling.

The effect of diagnostic sensitivity on the probability of detecting PRRSV infection in a single barn was evaluated for fixed spatial sampling as a function of sample size and number of positive pens (Table 3). Diagnostic specificity was assumed to be 100% for each level of diagnostic sensitivity. Conversely, the effect of diagnostic specificity on the probability of producing a false positive result is given in Table 4.

The effect of β_1 and β_2 on the probability of detection is shown in Fig. 2 for 2, 4, and 6 samples collected using fixed spatial sampling from one barn. Three separate scenarios were analyzed: one-half the observed spread of infection ($0.5 \times (\beta_1, \beta_2)$), the observed spread of infection ($1.0 \times (\beta_1, \beta_2)$), and twice the observed spread of infection ($2.0 \times (\beta_1, \beta_2)$). The number of positive pens by week were derived from the simulations and, therefore, vary slightly from the field data reported in Table 3. Table 5 reports the probability of ≥1 true positive results in 1, 2, or 3 barns as a function of the spread of infection (β_1, β_2), the number of barns sampled, the number of pens sampled within barns using a fixed spatial sampling, and the number of positive pens in the barns. The probabilities for 2 or 3 barns reported in Table 5 were calculated using Eq. (4).

4. Discussion

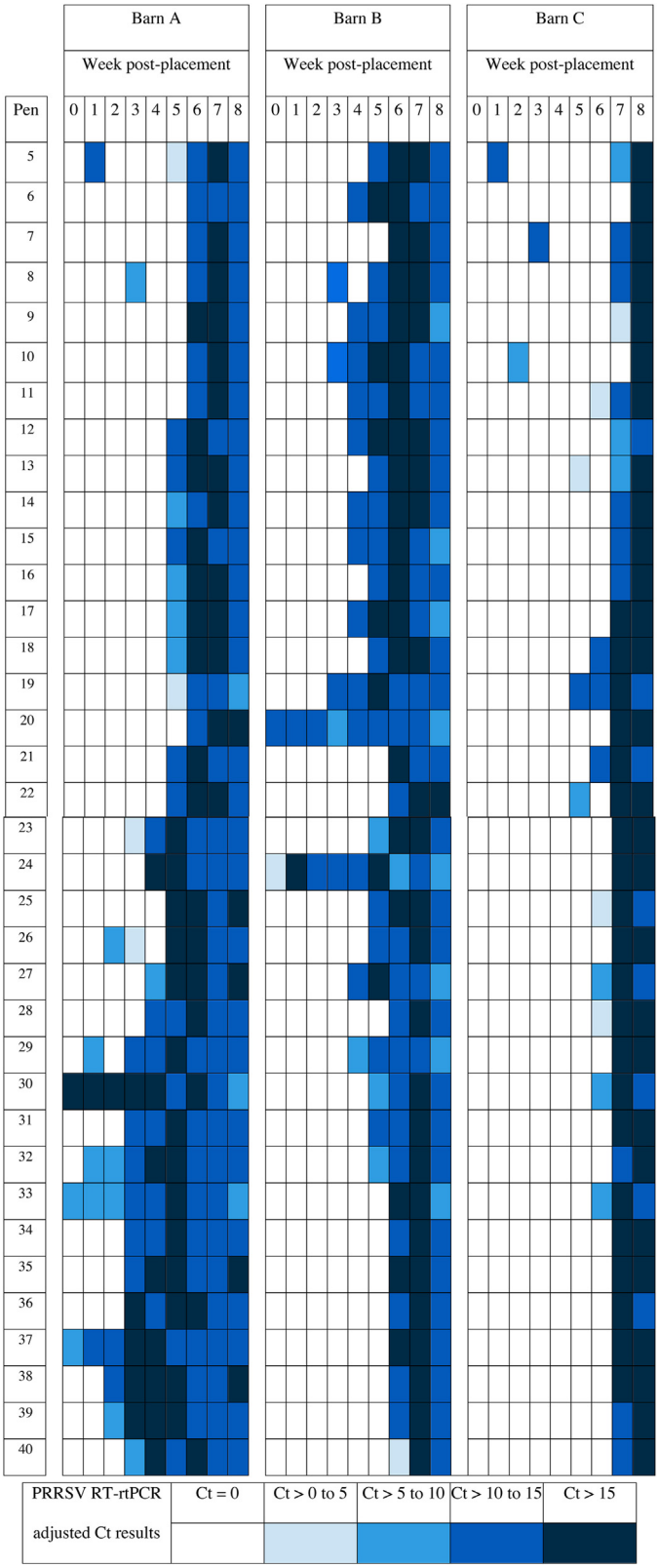
Cannon and Roe (1982) introduced the concept of statistical sampling to an earlier generation of livestock health specialists by presenting sample size guidelines based on perfect tests in a highly readable and widely disseminated pamphlet. The first wholesale application of statistical sampling to the livestock industry may have been the U.S. Aujeszky's disease (Pseudorabies) eradication program initiated in 1989 and successfully concluded in 2002 (Anderson et al., 2008). Subsequently, Cameron and Baldock (1998) developed formulas to calculate sample sizes for surveillance based on imperfect diagnostic tests and Cannon (2001) derived fast approximation formulas for this calculation. Such work provided a strong theoretical basis for surveillance based on individual animal samples, e.g., serum, but did not provide guidance for surveillance based on aggregate samples, e.g., oral fluids.

In this study, a piecewise exponential survival model was used to model 'time-to-infection' at the pen level using PRRSV RT-rtPCR results on oral fluid samples collected weekly (1, 2, ... t). Since sampling occurred at seven-day intervals, pen-level 'time-to-infection' was treated as interval-censored. The piecewise exponential model has previously been used for interval-censored time-to-event data where a constant hazard is assumed in each time interval. Covariate effects, if present, can be accommodated using proportional hazards (Friedman, 1982; Lindsey and Ryan, 1998). Simulation studies were then used to determine the effect of sampling allocation (simple random sampling vs. fixed spatial sampling), sample size, prevalence, time, and test performance (diagnostic sensitivity and specificity) on the probability of PRRSV detection in a single barn.

Independent of test performance, the probability of detection increased as sample size and/or PRRSV prevalence increased (Table 3); whereas, the probability of false positive results increased with larger sample size and/or with declining prevalence (Table 4). The overall trends observed were generally as expected, with estimates for specific conditions provided by the simulation studies.

Somewhat unexpectedly, fixed spatial sampling was found to be equal to, or better than, simple random sampling in terms of the probability of detecting infection (Fig. 1). Simple random sampling assumes that the characteristic of interest is independent and

Table 1
Spatiotemporal patterns of PRRSV spread in three wean-to-finish barns as revealed by weekly RT-rtPCR testing of pen-based oral fluids beginning one week post-weaning.



*Adjusted Ct is calculated as follows: Cutoff–Result = Adjusted Ct. Example: 45–30 = 15. The higher the adjusted Ct, the higher the concentration of virus detected.

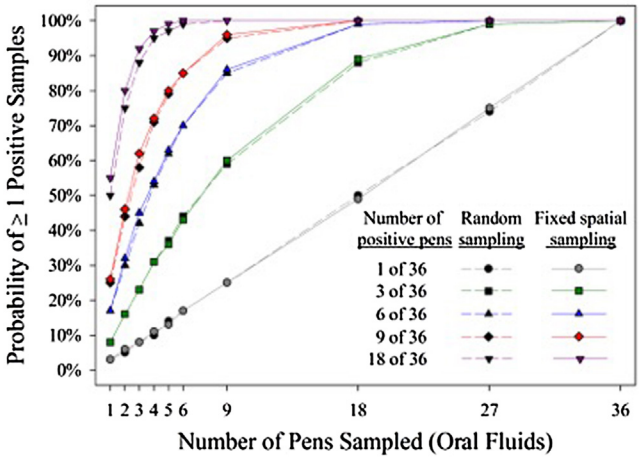


Fig. 1. Probability of detecting PRRSV in a single barn using pen-based oral fluids tested by RT-rtPCR as a function of sample allocation (simple random sampling vs. fixed spatial sampling), sample size, and prevalence.

spatially distributed (Cochran, 1977), but in infectious diseases, observations in proximity with each other are likely to be of similar status as a result of pathogen spread. Although rarely used in veterinary medicine, spatially-based sampling is widely used in other fields, where it is considered to offer advantages in terms of cost and efficiency (Wang et al., 2013). Fixed spatial sampling provides for a surveillance sampling design that is easily described and easily implemented in pig barns. Results of repeated sampling from the same pens over time provide a coherent picture of the infectious process and/or immune responses that can be easily juxtaposed with temporal productivity or clinical parameters.

Table 2
Model parameter estimates, standard errors, and 95% credible intervals.

	p_0	β_0	β_1	β_2
Estimate	0.032	3.980	−0.063	−1.286
Standard error	0.0167	0.3118	0.0427	0.0962
95% credible interval	[0.008, 0.073]	[3.339, 4.440]	[0.117, 0.031]	[−1.435, −1.082]

Currently, farm- or herd-level surveillance is challenged by the larger population size and heterogeneous hierarchies (sites, barns, animals) common to contemporary production sites. A design based on sampling individual barns provides flexibility in tailoring surveillance to farms ranging widely in size and complexity. Furthermore, sampling across multiple barns on a site is a powerful approach for detecting infection. For example, assuming fixed spatial sampling, within-barn prevalence of 25%, and test sensitivity/specificity of 95/100%, the probability of detecting PRRSV infection in one barn using 2 oral fluid samples is 43%

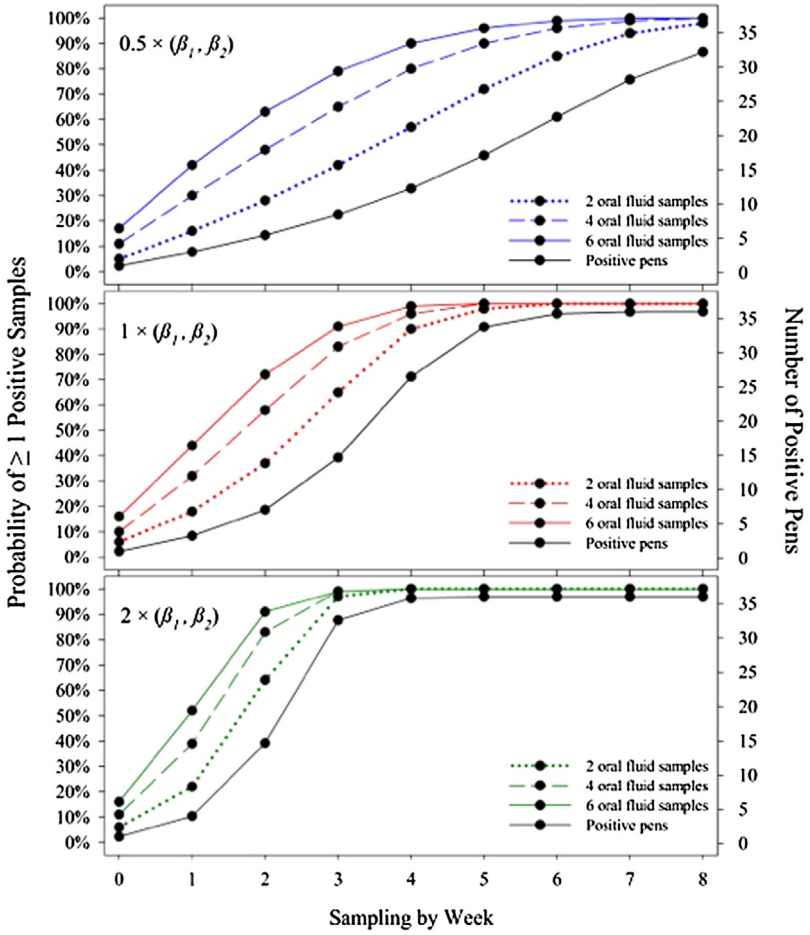


Fig. 2. Effect of spread of infection on the probability of detection by time in a single barn modeled by changing the values of β_1, β_2 in simulation studies. Fixed spatial sampling was used with sample sizes 2, 4, and 6 while allowing prevalence to change over time. For simplicity, diagnostic sensitivity and specificity were assumed to be 100%.

Table 3
Probability of ≥1 true positive results in one barn at one sampling. Probability (%, *in italics*) is a function of the number of positive pens in the barn, the number of pens sampled using a fixed spatial approach, and test diagnostic sensitivity[a].

Test	No. of samples	Number of positive pens among a total of 36 pens in the barn									
		1	2	3	4	5	6	9	18	27	36
Diagnostic sensitivity 60%	1	*2*	*3*	*5*	*7*	*9*	*10*	*16*	*34*	*52*	*60*
	2	*3*	*7*	*10*	*14*	*16*	*20*	*30*	*56*	*74*	*84*
	3	*5*	*10*	*15*	*20*	*24*	*29*	*40*	*70*	*86*	*93*
	4	*6*	*12*	*19*	*25*	*30*	*36*	*50*	*80*	*93*	*97*
	5	*9*	*17*	*23*	*31*	*37*	*42*	*58*	*86*	*96*	*99*
	6	*10*	*19*	*28*	*36*	*43*	*50*	*66*	*91*	*98*	*100*
	9	*15*	*28*	*39*	*49*	*56*	*64*	*80*	*97*	*100*	*100*
	18	*29*	*51*	*66*	*77*	*84*	*89*	*97*	*100*	*100*	*100*
	27	*46*	*70*	*83*	*91*	*96*	*98*	*100*	*100*	*100*	*100*
	36	*60*	*83*	*94*	*97*	*99*	*100*	*100*	*100*	*100*	*100*
Diagnostic sensitivity 70%	1	*2*	*4*	*6*	*8*	*10*	*13*	*19*	*38*	*60*	*69*
	2	*4*	*8*	*11*	*16*	*19*	*23*	*33*	*63*	*82*	*91*
	3	*6*	*12*	*17*	*22*	*27*	*32*	*46*	*76*	*92*	*97*
	4	*8*	*15*	*23*	*28*	*34*	*40*	*57*	*87*	*97*	*99*
	5	*10*	*19*	*27*	*35*	*42*	*49*	*65*	*92*	*98*	*100*
	6	*10*	*22*	*31*	*41*	*49*	*55*	*71*	*95*	*99*	*100*
	9	*17*	*32*	*45*	*56*	*64*	*72*	*86*	*99*	*100*	*100*
	18	*35*	*58*	*73*	*84*	*90*	*94*	*99*	*100*	*100*	*100*
	27	*54*	*77*	*90*	*95*	*98*	*99*	*100*	*100*	*100*	*100*
	36	*70*	*91*	*97*	*99*	*100*	*100*	*100*	*100*	*100*	*100*
Diagnostic sensitivity 80%	1	*3*	*4*	*7*	*9*	*11*	*14*	*21*	*46*	*69*	*80*
	2	*4*	*9*	*13*	*17*	*21*	*26*	*38*	*70*	*90*	*96*
	3	*7*	*13*	*19*	*25*	*31*	*36*	*52*	*82*	*97*	*99*
	4	*9*	*17*	*25*	*33*	*40*	*46*	*63*	*91*	*99*	*100*
	5	*11*	*21*	*31*	*39*	*47*	*54*	*71*	*95*	*100*	*100*
	6	*13*	*25*	*36*	*45*	*53*	*61*	*78*	*98*	*100*	*100*
	9	*20*	*37*	*50*	*61*	*70*	*77*	*90*	*100*	*100*	*100*
	18	*42*	*65*	*80*	*88*	*94*	*97*	*100*	*100*	*100*	*100*
	27	*60*	*84*	*93*	*98*	*99*	*100*	*100*	*100*	*100*	*100*
	36	*80*	*96*	*99*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Diagnostic sensitivity 90%	1	*2*	*5*	*8*	*10*	*13*	*15*	*24*	*50*	*78*	*90*
	2	*5*	*10*	*15*	*20*	*25*	*29*	*42*	*77*	*95*	*99*
	3	*7*	*15*	*22*	*29*	*35*	*41*	*57*	*89*	*98*	*100*
	4	*10*	*20*	*28*	*36*	*44*	*51*	*67*	*95*	*100*	*100*
	5	*12*	*24*	*34*	*43*	*52*	*59*	*76*	*98*	*100*	*100*
	6	*14*	*28*	*40*	*49*	*59*	*66*	*83*	*99*	*100*	*100*
	9	*23*	*41*	*55*	*66*	*74*	*82*	*94*	*100*	*100*	*100*
	18	*45*	*71*	*85*	*92*	*97*	*98*	*100*	*100*	*100*	*100*
	27	*68*	*90*	*97*	*99*	*100*	*100*	*100*	*100*	*100*	*100*
	36	*89*	*99*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Diagnostic sensitivity 95%	1	*3*	*5*	*8*	*10*	*13*	*16*	*25*	*53*	*81*	*95*
	2	*5*	*11*	*16*	*20*	*25*	*30*	*43*	*79*	*96*	*100*
	3	*8*	*16*	*23*	*30*	*36*	*42*	*58*	*90*	*99*	*100*
	4	*11*	*21*	*30*	*39*	*46*	*53*	*71*	*96*	*100*	*100*
	5	*12*	*25*	*36*	*46*	*55*	*63*	*80*	*99*	*100*	*100*
	6	*15*	*30*	*42*	*52*	*62*	*69*	*85*	*99*	*100*	*100*
	9	*23*	*43*	*58*	*68*	*77*	*84*	*95*	*100*	*100*	*100*
	18	*47*	*73*	*87*	*94*	*97*	*99*	*100*	*100*	*100*	*100*
	27	*73*	*93*	*98*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
	36	*94*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Diagnostic sensitivity 98%	1	*2*	*5*	*8*	*11*	*14*	*17*	*26*	*54*	*83*	*98*
	2	*5*	*10*	*16*	*22*	*26*	*31*	*45*	*81*	*97*	*100*
	3	*7*	*15*	*23*	*30*	*36*	*42*	*60*	*91*	*100*	*100*
	4	*12*	*21*	*30*	*39*	*46*	*54*	*71*	*97*	*100*	*100*
	5	*14*	*26*	*37*	*47*	*46*	*62*	*79*	*99*	*100*	*100*
	6	*16*	*30*	*42*	*53*	*62*	*69*	*85*	*99*	*100*	*100*
	9	*24*	*43*	*59*	*70*	*78*	*85*	*96*	*100*	*100*	*100*
	18	*49*	*74*	*88*	*94*	*98*	*99*	*100*	*100*	*100*	*100*
	27	*74*	*94*	*99*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
	36	*98*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Diagnostic sensitivity 100%	1	*3*	*5*	*8*	*12*	*14*	*17*	*26*	*55*	*83*	*100*
	2	*6*	*11*	*16*	*22*	*27*	*32*	*46*	*80*	*98*	*100*
	3	*8*	*16*	*23*	*30*	*38*	*45*	*62*	*92*	*100*	*100*
	4	*11*	*21*	*31*	*40*	*47*	*54*	*72*	*97*	*100*	*100*
	5	*13*	*26*	*36*	*46*	*55*	*63*	*80*	*99*	*100*	*100*
	6	*17*	*31*	*43*	*54*	*63*	*70*	*85*	*100*	*100*	*100*
	9	*25*	*45*	*60*	*72*	*80*	*86*	*96*	*100*	*100*	*100*

Table 3 (*Continued*)

Test	No. of samples	Number of positive pens among a total of 36 pens in the barn									
		1	2	3	4	5	6	9	18	27	36
	18	*49*	*75*	*89*	*95*	*98*	*99*	*100*	*100*	*100*	*100*
	27	*75*	*94*	*99*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
	36	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*

[a] Data for Table 3 were derived from the field data (Table 1) and simulation studies described in Section 2.5.1. Field data were derived from barns with 36 pens. Diagnostic specificity was assumed to be 100% to generate the data in Table 3.

(Table 3). Under these same assumptions, if 2 oral fluid samples were collected from each of 3 barns on one site, the probability of detection is 81%. This may be calculated using Equation (4): $P = (1 - (1 - p)^k) = (1 - (1–0.43)^3) = 0.81$. If prevalence is thought to differ among barns on a site, Table 3 and Equation (3) can be used to estimate the probability of detection by sample size. This approach assumes independence among barns. If this assumption does not hold, the piecewise exponential survival model can be generalized to include the pathogen's spread among barns and the overall chance of detection in multiple barns generated using simulations.

Sample size addresses the probability of detection at a single point in time, whereas the combination of sample size and frequency address the probability of detection as a pathogen spreads over time. The pattern of PRRSV spread observed in this study was in agreement with a previous report (Dufresne et al., 2003), but given that barns and pens-within-barns vary in design and size, it is possible that the parameters for the spread of infectious agents may differ somewhat among production sites. This concept has not been widely explored, but using a modeling approach, Maurice et al. (2016) predicted the spread of encephalomyocarditis virus to be faster in a barn with gated pens as opposed to concrete walls. The impact of spread on detection was addressed by modeling detection at 0.5, 1, and 2 times the observed spread of infection (Fig. 2, Table 5). From this analysis it can be seen that frequent sampling is mandatory, if early detection is the objective.

The first step in developing a sampling design is to establish a clear objective: surveillance vs. monitoring. To that end, the primary purpose of this study was to provide sampling guidelines for commercial pig farms. Given that perfect tests do not exist, a clear strategy for addressing unexpected results, e.g., suspected false positives, should be in place before sampling is initiated. Tables 3–5, provide the probabilities of detection for various scenarios and serve to guide sample size decisions. These tables describe the number of samples to collect in a barn as a function of the probability of detection. The number of pens in a barn is not an issue in selecting sample size. If the barn is designed with many

Table 4
Probability of ≥1 false positive results in one barn at one sampling. Probability (%, *in italics*) is a function of the number of positive pens in the barn, the number of pens sampled using a fixed spatial approach, and test diagnostic specificity[a].

Test	No. of samples	Number of negative pens among a total of 36 pens in the barn									
		1	2	3	4	5	6	9	18	27	36
Dx specificity = 98%	1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*2*
	2	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*	*3*	*4*
	3	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*3*	*4*	*6*
	4	*0*	*0*	*0*	*0*	*1*	*1*	*2*	*3*	*6*	*8*
	5	*0*	*0*	*0*	*1*	*1*	*2*	*2*	*4*	*7*	*9*
	6	*0*	*1*	*1*	*1*	*1*	*2*	*3*	*5*	*9*	*11*
	9	*0*	*1*	*2*	*2*	*3*	*4*	*5*	*8*	*13*	*17*
	18	*1*	*2*	*2*	*4*	*5*	*5*	*10*	*16*	*24*	*30*
	27	*1*	*2*	*3*	*4*	*5*	*7*	*11*	*22*	*34*	*43*
	36	*2*	*4*	*6*	*7*	*10*	*11*	*16*	*31*	*42*	*53*
Dx specificity = 99%	1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*
	2	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*	*2*
	3	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*	*2*	*3*
	4	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*3*	*4*
	5	*0*	*0*	*0*	*0*	*1*	*1*	*1*	*3*	*4*	*5*
	6	*0*	*0*	*0*	*1*	*1*	*1*	*1*	*3*	*4*	*6*
	9	*0*	*1*	*1*	*1*	*1*	*1*	*2*	*4*	*6*	*9*
	18	*0*	*1*	*2*	*2*	*3*	*3*	*4*	*8*	*12*	*16*
	27	*1*	*1*	*1*	*2*	*3*	*4*	*5*	*11*	*19*	*24*
	36	*1*	*2*	*3*	*3*	*5*	*6*	*8*	*16*	*24*	*31*
Dx specificity = 99.9%	1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	2	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	3	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	4	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	6	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*
	9	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*
	18	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*2*
	27	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*	*2*
	36	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*2*	*2*	*4*

[a] Data for Table 4 were derived from the field data (Table 1) and simulation studies described in Section 2.5.1. Field data were derived from barns with 36 pens. Diagnostic sensitivity was assumed to be 0% to generate the data in Table 4.

Table 5
Probability of ≥1 true positive results in 1, 2, or 3 barns. Probability (%, *in italics*) is a function of the spread of infection (β_1, β_2) the number of barns sampled, the number of pens sampled within barns using fixed spatial sampling, and the number of positive pens in the barn(s)[a].

	Sampling		Number of positive pens predicted over time (week)								
	Barns	Pens	1 (0)	3 (1)	5 (2)	8 (3)	12 (4)	17 (5)	23 (6)	28 (7)	32 (8)
Spread = 0.5 × (β_1, β_2)	1	2	*5*	*16*	*28*	*42*	*57*	*72*	*85*	*94*	*98*
		4	*11*	*30*	*48*	*65*	*80*	*90*	*96*	*99*	*100*
		6	*17*	*42*	*63*	*79*	*90*	*96*	*99*	*100*	*100*
	2	2	*10*	*29*	*48*	*66*	*82*	*92*	*98*	*100*	*100*
		4	*21*	*51*	*73*	*88*	*96*	*99*	*100*	*100*	*100*
		6	*31*	*66*	*86*	*96*	*99*	*100*	*100*	*100*	*100*
	3	2	*14*	*41*	*63*	*80*	*92*	*98*	*100*	*100*	*100*
		4	*30*	*66*	*86*	*96*	*99*	*100*	*100*	*100*	*100*
		6	*43*	*80*	*95*	*99*	*100*	*100*	*100*	*100*	*100*
	Barns	Pens	1 (0)	3 (1)	7 (2)	15 (3)	26 (4)	34 (5)	36 (6)	36 (7)	36 (8)
Spread = 1.0 × (β_1, β_2)	1	2	*6*	*18*	*37*	*65*	*90*	*98*	*100*	*100*	*100*
		4	*10*	*32*	*58*	*83*	*96*	*100*	*100*	*100*	*100*
		6	*16*	*44*	*72*	*91*	*99*	*100*	*100*	*100*	*100*
	2	2	*12*	*33*	*60*	*88*	*99*	*100*	*100*	*100*	*100*
		4	*19*	*54*	*82*	*97*	*100*	*100*	*100*	*100*	*100*
		6	*29*	*69*	*92*	*99*	*100*	*100*	*100*	*100*	*100*
	3	2	*17*	*45*	*75*	*96*	*100*	*100*	*100*	*100*	*100*
		4	*27*	*69*	*93*	*100*	*100*	*100*	*100*	*100*	*100*
		6	*41*	*82*	*98*	*100*	*100*	*100*	*100*	*100*	*100*
	Barns	Pens	1 (0)	4 (1)	15 (2)	33 (3)	36 (4)	36 (5)	36 (6)	36 (7)	36 (8)
Spread = 2.0 × (β_1, β_2)	1	2	*6*	*22*	*64*	*97*	*100*	*100*	*100*	*100*	*100*
		4	*11*	*39*	*83*	*99*	*100*	*100*	*100*	*100*	*100*
		6	*16*	*52*	*91*	*99*	*100*	*100*	*100*	*100*	*100*
	2	2	*12*	*39*	*87*	*100*	*100*	*100*	*100*	*100*	*100*
		4	*21*	*63*	*97*	*100*	*100*	*100*	*100*	*100*	*100*
		6	*29*	*77*	*99*	*100*	*100*	*100*	*100*	*100*	*100*
	3	2	*17*	*53*	*95*	*100*	*100*	*100*	*100*	*100*	*100*
		4	*30*	*77*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
		6	*41*	*89*	*100*	*100*	*100*	*100*	*100*	*100*	*100*

[a] Data for Table 5 were derived from the field data (Table 1) and simulation studies described in Section 2.5.2. The probabilities for 2 and 3 barns were calculated using Eq. (4). Diagnostic sensitivity and specificity were assumed to be 100%.

pens, samples will likely be collected from separate pens. If the barn is designed with few pens, more than one sample per pen could be collected. The key feature is a fixed spatial approach: space samples equally over the length of the barn.

The purpose of surveillance is to assure animal health and welfare, improve producer profitability, and protect a valuable national asset. The specific objective of the present study was to develop sampling guidelines for oral fluid-based PRRSV surveillance or monitoring in commercial swine farms. These results will have immediate application to PRRSV control and/or elimination projects at the herd, area, and regional levels. The analysis was based on PRRSV infection in commercial swine production facilities detected using PRRSV RT-rtPCR testing, but the results are expected to be broadly applicable to swine pathogens for which oral fluid tests of equivalent performance are available.

Funding

This study was funded in part by Checkoff Dollars administered through the National Pork Board (Grant #13-157), Des Moines, Iowa (USA).

Acknowledgement

Our thanks to Sarah Bade at the Iowa State Diagnostic Laboratory for performing PRRSV RT-rtPCR testing.

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