Surveillance on swine farms using antemortem specimens

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1 Introduction

Surveillance is the process of tracking health and productivity parameters over time to understand population health dynamics and to make better decisions on disease control. To be thorough, we note that monitoring and surveillance are not quite the same. Specifically, monitoring is the systematic collection and evaluation of population data over time, whereas surveillance is monitoring along with a plan prepared and ready for implementation if a specific threshold or disease condition is identified (Salman, 2003). Although they are not synonymous, monitoring and surveillance are generally used interchangeably in daily life and, for simplicity, in this review (Paskins, 1999).

The aim of this chapter is to provide a general, non-mathematical overview of infectious disease surveillance on swine farms based on testing. Because farms vary in size, structure, management, and surveillance goals, there is no 'one-size-fits-all' surveillance plan that can fit all circumstances and meet all objectives. Rather, the design and implementation of a surveillance program should be driven by the producer, ideally working in conjunction with an animal health specialist, and tailored to meet the specific objectives of the production system. Bedrock principles should guide the design process,

which should be periodically reviewed after the surveillance program is initiated:

- Surveillance objective(s) should be clear and shared by all involved.
- The process – from sample collection to data interpretation – should be simple, clearly understood, and easily performed.
- The process should produce timely, accurate, interpretable and actionable results.
- The process must provide a return on investment through the reduction or avoidance of disease losses and/or enhancement of the value of the product.
- The process should be adaptable and able to meet new objectives as they are identified.

2 Overview

Representative sampling, testing a subset of randomly selected individuals to establish the status of the entire population, was the first step toward efficient surveillance. First described in 1895 (Kruskal and Mosteller, 1980), statistical sampling was rarely used in livestock surveillance until a synopsis by Cannon and Roe (1982) made the concepts accessible and understandable to field veterinarians. Subsequently, surveillance sample sizes based on binomial sampling distributions were routinely designed into swine disease control programs, for example, the U.S. pseudorabies (Aujeszky's disease) eradication program (Anderson et al., 2008), and became an integral part of the thought processes of swine health specialists.

The two key assumptions underlying binomial sampling are (1) the population is homogeneous, that is, randomly selected pigs in the population have an equal chance of being positive, and (2) the pigs in the population are 'independent,' that is, the infectious disease status of one pig is not predictive of the status of another (Wroughton and Cole, 2013). These assumptions were sometimes true in the smaller herds of the past, but are rarely true today because pigs on commercial production sites are separated into buildings, rooms, and pens by age, production stage, and/or function, with little interaction between groups. The result is the heterogeneous distribution (clustering) of disease within a production site. That is, some groups may be positive and others negative for the pathogen of interest, on the same farm at the same point in time. In addition, because infectious agents are most commonly spread from pig to pig, pigs in the same pen or barn are likely to be of similar status (Rotolo et al., 2017). It follows that, because pigs in physical proximity are likely to share the same disease status, they are not independent.

To account for this population structure and the non-random disease distribution, at least to the degree possible, it is useful to design surveillance based on 'epidemiological units,' that is, groups of animals on the site that share a common environment and/or a comparable risk of exposure to the pathogen of interest (OIE, 2021). For example, in the U.S. pseudorabies (Aujeszky's disease) eradication program, '*each segregated group of swine on an individual premises* … (was) *considered a separate herd.*' Thus, according to the program guidelines, a farm could consist of one or more 'herds' (epidemiological units). Regardless of the number, each 'herd' was sampled according to the official (binomial sampling) protocol, for example, 29 pigs were sampled in each barn holding ≥ 1000 animals to achieve a 95% probability of detection at 10% prevalence (USDA, 2003).

A further complication to surveillance is the continual turnover of animals on swine farms. The production cycle is short for both market pigs (six months from birth to market) and breeding stock; that is, the turnover in finishing barns may approach 250% per year and breeding herds replace 40–50% of females annually (Stalder et al., 2004). As a point of contrast, human population turnover in 28 European countries for 2016 ranged from a low of 2.4% in Italy to a high of 8.5% in Luxembourg (Eurostat News, 2017). Further, as animals complete the production cycle, replacement animals are introduced, either through birth or from other farms. If replacements are immunologically susceptible to an infectious agent on the farm, they will eventually become infected and perpetuate the pathogen on the farm. If new replacements are infected with a pathogen not present on the farm, the risk is that it will spread to the remainder of the herd. This is a common scenario, that is, moving animals between herds is the most frequent route of PRRSV spread (Pileri and Mateu, 2016). Thus, sampling and testing must be sufficiently frequent in order to accommodate the rate of population turnover and the continual introduction of replacements.

3 Collecting production data

Pig producers have long recognized the value of data. For example, British pig producers in the 1920s used records to identify prolific sows that produced fast-growing, early-maturing progeny with good carcass characteristics (Woods, 2012). With the appearance of specialized pig farms in the mid-twentieth century, the goals of surveillance were broadened to include other health and productivity parameters (Alexander, 1971; Muirhead, 1976). This was, in part, a response to new disease challenges. That is, indoor housing alleviated health and welfare issues associated with outdoor pig production by providing better parasite control, nutrition and protection against extreme weather. However, confinement also changed the ecological balance among pigs, pathogens, and their environment and, in some circumstances, led to

the emergence of new multifactorial disease syndromes (Muirhead, 1976; Woods, 2012). This problem remains with us, a contemporary example being the porcine respiratory disease complex (PRDC), '*a combination of primary and opportunistic infectious agents, often facilitated by adverse environmental and management conditions*' (Yaeger and Van Alstine, 2019). Responding to the challenge, swine health innovators of the time recommended a new approach based on the systematic collection and analysis of farm data to establish herd baselines, unravel complex disease causalities, and evaluate the effects of management decisions on health and productivity (Muirhead, 1976; Schwabe, 1982; Stein et al., 1987). Working at the cusp of this transition, Stein et al. (1987) aptly described this process: '... *just as the stethoscope and thermometer are fundamental tools for individual medicine, production and health recording systems are fundamental tools for effective population medicine.*'

Production and health recording systems relied on hand-written records into the 1970s and later, but producers readily adopted electronic data management as computer technology became accessible. Thus, Pepper et al. (1977a) described the use of a Fortran computer program for the analysis of reproductive parameters. On the farm, the data were written on 'sow cards.' The cards were then taken to a computer center for data entry and analysis, for example, litters per sow per year, weaning-to-service intervals, etc. Pepper and Taylor (1977b) used this system, in combination with necropsies of all pigs that died between Sunday 9:00 am and Friday 9:00 am, to achieve major improvements in performance in a 260-sow breeding herd. Electronic production records became increasingly commonplace after affordable desktop computers became available in the mid-1980s and continuous improvements in information technology have brought us to the point where it is now possible to collect and analyze data across entire production systems with thousands of animals distributed among multiple production sites (Magalhães et al., 2022).

4 Collecting surveillance data

In contrast to systems for the collection of production data, the tools needed for practical infectious disease surveillance required breakthroughs in diagnostic medicine, which have only been realized in recent years. Among these developments, we will focus on tests and specimens compatible with live animal surveillance in commercial swine herds, that is, assays able to detect pathogen-specific antigen, antibody or nucleic acid in specimens collected from live pigs or their environment. Diagnostic technology continues in a state of ongoing development and a periodic review of new commercially available tests and their performance is advised for the sake of keeping up with innovations.

4.1 Testing technology

The first major breakthrough in testing came with the development of the enzyme-linked immunosorbent assay (ELISA) in 1971 (Engvall and Perlmann, 1971; Van Weemen and Schuurs, 1971). This was followed quickly by the development of a variety of pathogen-specific antibody ELISAs (Schuurs and Van Weemen, 1977). ELISAs can be designed to measure either antigen or antibody, but both use an enzyme to detect antigen–antibody binding. Essentially, in the presence of antigen–antibody binding, the enzyme converts a colorless enzyme substrate (chromogen) to a colored product. The strength of the color reaction is measured using a spectrophotometer, with the measurement (optical density) directly proportional (indirect ELISA) or inversely proportional (blocking and competitive ELISAs) to the concentration of the target in the sample. Currently, ELISA remains the most widely used antibody detection method because of its simplicity, low cost, consistent performance and wide commercial availability.

In addition to ELISAs, a variety of other immunoassay technologies have been reported, but most have not been commercialized. These assays take on a wide range of formats, but most consist of an antigen or antibody that is immobilized on a surface (planar or microbead-based) and binds virus-specific antigens or antibodies present in a sample. By adding a reporter protein, it is possible to detect a pathogen-specific immune signal to confirm the presence of pathogen-specific antibody. Many of these systems show promise, point-of-care tests being one example. However, they need to meet regulatory, test performance and commercial expectations (Hobbs et al., 2021).

In surveillance, the role of antibody testing is to detect infection with a pathogen (in the absence of vaccination), track an outbreak over time, assess the response to vaccination or controlled exposure and/or assess the level of protective immunity within a population (Arnold and Chung, 2018; Cutts and Hanson, 2016; MacNeil et al., 2014). Depending on the agent, pathogen-specific antibody is usually detectable one to three weeks post infection, and thereafter for months to years in various diagnostic specimens, for example, serum, oral fluids, feces, mammary secretions or tissue exudate ('meat juice,' processing fluid). Notably, maternal antibody or antibody induced by vaccines cannot usually be differentiated from wild-type infections, with the exception of antibody induced by DIVA (differentiating infected from vaccinated individuals) vaccines (van Oirschot, 1999). DIVA vaccines induce protective immunity. However, they are missing one or more epitopes present in the wild-type pathogen. Thus, DIVA-vaccinated animals are negative for specific antibodies that are present in wild-type-infected animals. Pseudorabies virus DIVA vaccines and the accompanying ELISA made pseudorabies virus control and elimination possible (Mettenleiter, 2020).

The second major diagnostic breakthrough came with the publication of the principles underlying the polymerase chain reaction (PCR) (Saiki et al., 1985). As with the ELISA, PCRs were quickly adapted to swine diagnostic medicine, with pseudorabies virus DNA detection being among the first examples of its use (Belak et al., 1989; Jestin et al., 1990; Maes et al., 1990). PCR is based on the amplification of nucleic acid into quantities that can be detected and analyzed. PCR begins with the addition of short DNA sequences (primers) to denatured (single-stranded) DNA. Except that, in the case of RNA viruses, PCR is preceded by a step to produce a complementary DNA template (cDNA) from viral RNA by the addition of a reverse transcriptase enzyme. Binding of primers to the single-stranded DNA forms double-stranded DNA and triggers a thermostable polymerase enzyme to extend the sequence and produce a full, complementary strand of DNA. End-point methods then measure the amount of amplified product ('amplicon') accumulated over the course of the reaction. The development and commercialization of fluorometry-based real-time PCR, that is, quantitative real-time PCR (qPCR or RT-qPCR for RNA viruses), improved and simplified the process of amplifying and detecting nucleic acid sequences while allowing for simultaneous and more precise quantification of their concentrations (Klein, 2002). Thus, samples with a higher concentration of target nucleic acid will require fewer PCR amplification cycles to reach the cycle threshold (Ct) established as the assay cutoff for detection (Schmittgen and Livak, 2008).

Depending on the pathogen, detectable levels of pathogen-specific nucleic acid may be present hours to days after infection in various diagnostic specimens, for example, serum, oral fluids, feces, mammary secretions, or tissue exudate ('meat juice,' processing fluid). The ability of PCR to detect acute infections makes it particularly useful in verifying freedom from infection in animals scheduled for upcoming movement. PCR is also compatible with testing environmental samples, for example, surfaces, water and air, but it should be borne in mind that current qPCRs cannot differentiate between viable and non-viable (inactivated) pathogens. That is, a positive result indicates the presence of pathogen-specific genetic material, but not its viability or infectivity.

Although not strictly a breakthrough in diagnostic technology, the third significant development in surveillance was the creation of systems for managing, storing and analyzing testing data. In top-tier veterinary diagnostic laboratories, this would include laboratory information management systems (LIMS) that are capable of tracking samples, retrieving/storing electronic output from test devices, and reporting results to clients in any of the various electronic formats. In smaller laboratories or in on-farm testing, this may only include receiving results as electronic data. Regardless, these technologies have played a major role in improved throughput and timely data analysis/response.

4.2 Samples and specimens

The antemortem specimens commonly used in surveillance are listed in Tables 1 and 2 for specific bacterial and viral pathogens, respectively. These tables are intended to provide an overview and should not be considered comprehensive. To begin this section, a brief overview of sampling terminology may be useful.

'Discrete' samples are defined as samples collected at one point in time from a specific source and location (Cameron et al., 2003; Patil et al., 2010). Without time and place identifiers, it is not possible to interpret test results in the context of the population under surveillance. Discrete samples may be specimens from one animal, for example, serum, nasal swabs, etc., or simultaneously collected from more than one animal ('aggregate' samples), for example, pen-based oral fluids or environmental samples.

Two or more discrete samples may be combined (composite or 'pooled' samples) into one for testing. The purpose of pooling is to reduce the number of test samples and, thereby, reduce cost. Pooling is not a new idea. For example, Dorfman (1943) tested pooled serum samples when screening World War II draftees for syphilis. The concept of detection at a lower cost is highly alluring and there is an extensive body of literature on the subject of pooling (Daniel et al., 2021). However, pooling should be used cautiously because it increases the rate of false negative results. That is, combining negative and positive samples must necessarily dilute the concentration of the test target in the pool. The risk is diluting the sample to a concentration below the threshold of detection. For example, Rovira et al. (2007) compared the detection of PRRSV in blood swab and serum samples during acute infection (1 to 15 days post inoculation) and found that pooling in groups of five resulted in 6% fewer RT-qPCR-positive serum samples and 8% fewer positive blood swab samples compared to testing the samples individually. Ultimately, the decision to pool (or not) depends on the cost of false-negative results versus the savings afforded by performing fewer tests. If pooling is done, it should be done in such a way as to preserve sampling location and date integrity. That is, pooling samples from different locations or dates will produce uninterpretable results.

4.2.1 Blood-derived specimens

Commonly used blood-derived specimens include whole blood, serum and blood swabs. Methods for collecting blood samples from pigs are described elsewhere (Ramirez and Karriker, 2019). Pig blood coagulates quickly, and when collecting whole blood, the sample should be collected directly into a vacutainer blood-collection tube containing an anticoagulant. Djordjevic et al. (2006) reported that sodium heparin inhibited PCR amplification, but other anticoagulants did not, that is, sodium citrate, K3 EDTA and lithium heparin.

Table 1 An overview of antemortem specimens commonly used in surveillance for bacterial pathogens (numbers correspond to references listed in Section 9)

Bacterial pathogens	Assay Target[a]	Serum	Whole blood	Blood swab	Oral fluid	Oral swab	Nasal swab	Oropharyngeal swab	Tonsil scraping	Tracheal swab	Feces	Rectal swab	Processing fluids	Colostrum/ milk	Vaginal swab	Semen	Environmental
Actinobacillus pleuropneumoniae	AB	295	-	-	103	159	25	-	-	-	-	-	-	197	-	-	-
	NA	-	-	-	44	-	274	178	274	-	-	-	-	-	-	-	158
Actinobacillus suis	AB	145	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bordetella bronchiseptica	AB	131	-	-	-	-	141	-	-	-	-	-	-	215	-	-	-
	NA	-	-	-	177	-	177	-	-	-	-	-	-	-	-	-	-
Brachyspira spp.	AB	147	-	-	293	-	-	-	-	-	176	-	-	-	-	-	-
	NA	-	-	-	-	-	-	-	-	-	125	214	-	-	-	-	95
Brucella suis	AB	273	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	AB	228	-	-	-	24	-	-	-	-	280	-	-	227	-	-	-
	NA	-	-	-	-	-	-	-	-	-	264	119	-	-	-	-	139
Erysipelothrix rhusiopathiae	AB	98	-	-	99	-	-	-	-	-	-	-	-	126	-	-	-
	NA	-	-	-	99	98	-	-	-	-	-	-	-	-	-	-	110
Glaesserella parasuis	AB	185	-	-	-	89	203	-	-	-	-	-	-	164	-	-	-
	NA	-	-	-	44	-	11	168	-	-	-	-	-	-	-	-	157
Mycoplasma hyopneumoniae	AB	79	-	-	114	151	80	-	-	-	151	-	23	235	-	-	-
	NA	-	-	-	-	-	31	78	78	78	279	-	-	-	-	-	266
Lawsonia intracellularis	AB	137	-	-	16	-	-	-	-	-	53	-	-	-	-	-	-
	NA	-	-	-	86	-	-	-	-	-	137	192	-	-	-	-	-
Mycoplasma hyorhinis	AB	174	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NA	96	-	-	44	-	51	243	-	-	-	-	-	-	-	-	-

Mycoplasma hyosynoviae	AB	109	-	-	-	-	-	-	-	-	-	146	-	-
	NA	96	-	-	96	-	243	102	-	-	-	-	-	-
Mycoplasma suis	AB	117	116	-	-	-	-	-	-	-	-	-	-	-
	NA	-	-	69	-	69	-	-	-	-	-	-	69	69
Pasteurella multocida	AB	196	-	-	-	-	-	-	275	-	-	196	-	-
	NA	-	-	129	44	-	129	-	-	-	-	-	-	157
Salmonella enterica	AB	194	-	-	14	63	-	-	-	-	124	23	-	-
	NA	-	-	-	86	-	-	-	-	223	-	-	-	205
Streptococcus suis	AB	29	-	-	-	-	-	-	-	-	18	-	-	-
	NA	-	-	66	44	12	179	-	12	12	-	-	12	179

[a] AB = antibody NA = nucleic acid

Table 2 An overview of antemortem specimens commonly used in surveillance for viral pathogens (numbers correspond to references listed in Section 9)

Viral pathogens[a]	Assay Target[b]	Serum	Whole blood	Blood swab	Oral fluid	Oral swab	Nasal swab	Oropharyngeal swab	Tonsil scraping	Tracheal swab	Feces	Rectal swab	Processing fluids	Colostrum/ milk	Vaginal swab	Semen	Environmental
APPV	AB	229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NA	229	-	-	304	255	190	-	-	-	62	190	67	-	-	92	-
ASFV	AB	56	-	37	97	-	-	-	-	-	198	201	-	-	-	-	-
	NA	1	308	37	105	105	308	60	308	-	61	-	-	-	-	-	43
CSFV	AB	292	-	-	210	-	-	-	-	-	-	-	-	263	-	-	-
	NA	2	2	220	105	105	240	70	240	-	47	54	-	-	-	83	47
FMDV	AB	87	-	-	258	-	-	269	-	-	-	-	-	88	-	-	-
	NA	81	81	-	287	268	5	-	-	-	191	-	-	-	-	282	52
HEV	AB	288	-	-	-	-	-	-	-	-	-	-	68	73	-	-	-
	NA	132	-	-	225	-	181	-	-	-	82	40	-	-	-	149	15
IAV	AB	135	-	-	207	-	-	-	-	84	-	-	23	-	-	-	-
	NA	21	-	-	21	-	91	91	-	-	9	-	-	-	-	-	91
PCMV	AB	155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NA	188	188	-	-	187	74	-	-	-	290	187	-	-	-	154	-
PCV2	AB	36	-	-	232	-	-	-	-	-	-	-	160	93	-	-	-
	NA	27	259	204	195	297	259	259	252	256	259	85	160	108	239	204	163
PDCoV	AB	272	-	-	-	-	-	-	-	-	-	-	276	166	-	-	-
	NA	286	-	-	118	-	286	-	-	-	305	305	-	305	-	-	262
PEDV	AB	94	-	-	22	-	-	-	-	-	94	-	-	226	48	-	-
	NA	-	150	-	22	-	193	-	-	-	170	22	-	-	-	90	165
PPV	AB	57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NA	57	41	-	183	-	206	-	252	-	184	206	-	-	-	104	-

PRRSV	AB	3	-	217	231	217	-	-	-	-	113	-	160	242	217	-
	NA	50	123	217	231	217	46	65	153	45	294	-	160	-	217	250
PRV	AB	156	-	-	208	-	-	241	-	-	143	-	-	247	-	-
	NA	238	-	-	208	-	152	-	26	-	-	-	-	-	-	148
SVDV	AB	237	-	-	257	-	-	-	-	-	-	-	-	-	-	-
	NA	81	81	-	257	257	81	81	-	-	81	122	-	-	282	-
SVV	AB	28	-	-	-	-	-	-	-	-	-	-	-	301	-	-
	NA	28	-	-	42	128	28	306	-	-	34	28	-	-	271	299
TGEV	AB	272	-	-	175	55	-	-	-	-	-	-	-	144	-	-
	NA	-	-	-	175	-	-	-	-	-	133	211	-	-	-	-
VESV	AB	138	-	-	-	167	-	-	-	-	-	-	-	-	-	-
	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VSV	AB	186	-	-	-	-	-	-	-	-	-	-	-	130	-	-
	NA	186	284	-	-	-	186	186	-	-	81	-	-	-	-	-

a See abbreviations in Section 7.0.
b AB = antibody NA = nucleic acid

Serum, the liquid portion of clotted blood, is recovered by centrifugation of blood samples collected without an anticoagulant. Blood swabs, a major innovation for the detection of pathogens that produce a pronounced viremia, for example, PRRSV, are collected by puncturing a superficial vein with a needle, saturating the swab with the blood that pools at the site of the puncture, and then placing the swab into a tube containing normal saline (Reicks et al., 2006). Under some circumstances, blood swabs can be collected without animal restraint, and therefore, may be a practical alternative to standard blood collection methods in boars or adult pigs (Carlson et al., 2018).

4.2.2 Oral cavity and upper respiratory tract specimens

Oral fluid, originally reported for the detection of PRRSV-specific nucleic acid and antibody (Prickett et al., 2008a), is the liquid collected by placing an absorptive device in the buccal cavity. Oral fluids are collected from individual pigs or from a group of pigs by suspending a length of cotton rope in their pen and allowing them to chew it for 15–30 minutes. The sample is recovered by squeezing the rope after placing it in a plastic bag. The fluid that accumulates in the bottom of the bag is then poured into a tube. Oral fluids are easily collected, and as reviewed by Henao-Diaz et al. (2020a), nucleic acid and/or antibody have been reported in oral fluids for essentially all common pathogens of swine.

Oral swabs (aka buccal swabs) are collected from the rostral portion of the oral cavity, that is, inner cheek mucosa, tongue, teeth and gums. They have been used for the isolation of swine vesicular disease virus, *Campylobacter* spp., and foot-and-mouth disease virus (Callahan et al., 2002; Kodama et al., 1980; McOrist and Lawson, 1989). For sample collection, the pig's mouth is held open with an oral speculum and a sterile swab is inserted into the oral cavity. The swab is moved in circular motion in the mouth while trying to avoid feed or other contaminants and then placed into a tube containing the appropriate medium (Arai et al., 2018). Oral swabs are sometimes considered equivalent to oral fluid, but as reviewed by Henao-Diaz et al (2020a), the simple act of restraining the pig to collect an oral swab induces a stress response that results in vasoconstriction of vessels supplying the buccal tissues. This then reduces the flow of fluids to the mouth and alters the composition of buccal fluids. This explains why, in a direct comparison, Prickett et al. (2008a) reported a lower rate of detection in oral swabs vs oral fluid samples from the same animals.

Nasal swab samples were first reported in the late 1960s and early 1970s for the isolation of *Mycoplasma* spp., *Bordetella bronchiseptica*, parvovirus, cytomegalovirus and influenza A virus (Gois et al., 1969; Kemeny and Amtower, 1973; Pirtle, 1975; Watt, 1978). To collect a nasal swab sample, a sterile pre-moistened swab is inserted into one naris of the pig and rotated while avoiding contact with the outside of the nostril. This process is repeated in the other naris

with the same swab or with a new one and then the swab(s) is (are) placed in a tube containing an appropriate medium.

The caudal portion of the oral cavity, including the soft palate, throat, tonsils and the back of the tongue are an excellent sampling site because the tonsils are the site of primary replication and/or chronic persistent infection for many of the most troublesome bacterial and viral pathogens, for example, *Salmonella* spp., *Actinobacillus pleuropneumoniae*, PRRSV, foot-and-mouth disease virus and classical swine fever virus (Horter et al., 2003). Samples collected from the oropharynx include swabs, tonsil biopsies, and tonsil scrapings. Oropharyngeal swabs are collected by holding the pig's mouth open with an oral speculum, swabbing the oropharynx with a pre-moistened sterile swab, particularly targeting the tonsils, and placing the swab into a tube containing an appropriate medium. Biopsies of the tonsil of the soft palate were done routinely in the US 'hog cholera' eradication program (USDA, 1981), but the procedure requires considerable technical skill. Tonsil scrapings are a highly effective and less invasive alternative to tonsil biopsies. The sample is collected by scraping the tonsils of the soft palate with a stainless steel spoon while holding the pig's mouth open with an oral speculum. The material that pools in the bowl of the spoon is then collected with a sterile swab and placed in a tube containing the appropriate medium (Wills et al., 1997). Tracheal swabs were originally reported for the isolation of influenza A virus and later for the isolation of *Glasserella parasuis* and *Mycoplasma hyopneumoniae* (Carrou et al., 2006; Kirkwood et al., 2001; Shortridge and Webster, 1979). Tracheal swabs provide improved detection for some respiratory pathogens but the procedure requires technical skill (https://vetmed.iastate.edu/story/vdl -tracheal-sampling).

4.2.3 Miscellaneous specimens

Fecal samples are easily collected, but may present practical challenges due to stool consistency and/or the potential risk of contamination during handling (Choudhury et al., 2019). For fecal collection, restrain the pig, insert two fingers of a gloved hand into the rectum, recover the sample, and place it into a clean tube or plastic bag for testing or storage. Alternatively, fecal samples may be collected by inserting a sterile swab or fecal loop into the rectum and rotating gently against the bowel wall. The swab (or fecal loop sample) is then placed into a tube containing the appropriate medium. The specimens may serve for pathogen isolation, but false negatives can occur because the analyte concentration is lower than in the actual fecal samples (Choudhury et al., 2019).

Processing fluids are serosanguineous fluids recovered from testicles and tails collected at the time of piglet processing. They were originally proposed as a sow surveillance tool for the detection of PRRSV, influenza A virus, porcine

circovirus 2, porcine parvovirus, *Mycoplasma hyopneumoniae* and *Salmonella enterica* (Boettcher et al., 2010). This original observation has been supported by subsequent research (Campler et al., 2021; López et al., 2018; Vilalta et al., 2018).

Colostrum and milk were first used to evaluate antibody responses in dams immunized against influenza A virus (Young and Underdahl, 1950). For collection, udders are cleaned, the first ten spurts discarded, and the sample collected in clean tubes. Fat and debris may be removed by centrifugation (13 000 x *g* for 15 minutes), with the middle layer and pellet collected for testing (Ha et al., 2009; Poonsuk et al., 2016). Mammary secretions represent the infectious disease status of the dam and may be tested either for pathogens or for antibodies (Wagstrom et al., 2001).

The use of vaginal swabs includes reports of the isolation of African swine fever virus and *Brucella abortus* (Greig and Plowright, 1970; Stuart et al., 1987). Vaginal swabs are collected by introducing a sterile swab ~8 cm into the vaginal tract, rotating gently in a circular motion while holding against the vaginal wall, and then placing in a tube containing an appropriate medium (Pena Cortes et al., 2018). Care should be taken to avoid fecal contamination (Gresham, 2003).

Semen has been used for the detection of sexually transmitted pathogens such as *Brucella abortus* and PRRSV (Christopher-Hennings et al., 1995; Hutchings and Andrews, 1946), but it generally does not provide good test performance and other specimens should be given consideration (Christopher-Hennings et al., 1995; Maes et al., 2008). In addition, semen collection is a technical procedure that requires training both the personnel and the boar.

Environmental samples (air, water, surfaces and feedstuffs) are commonly reported in the literature and may be useful as surveillance samples. However, the limitations inherent in environmental samples are significant. That is, if present in the environment, (1) the concentration of target collected in the sample is often below the assay's limit of detection (Girones et al., 2010; Garrido-Mantilla et al., 2019) and (2) targets are often non-uniformly distributed (O'Connor et al., 2006). Thus, negative results may represent sampling/testing error, and positive results, if based on nucleic acid detection, may be difficult to interpret because the result may represent non-infectious material.

5 Test performance

Yerushalmy (1947) originally defined test performance in terms of sensitivity or specificity, but the introduction of PCR-based tests necessitated classifiers (Saah and Hoover, 1997):

- 'Diagnostic' sensitivity – the probability that a test on a sample from a positive source will produce a positive test result. Note that 'source' could be a specimen collected from an animal or from the environment (air, water, surfaces and feedstuffs).
- 'Analytical' sensitivity – an estimate of the lowest concentration of target that an assay will detect.
- 'Diagnostic' specificity – the probability that a test on a sample from a negative source will produce a negative test result.
- 'Analytical' specificity – a measure of a test's reactivity with non-target substances.

Among the four, diagnostic specificity is the most important parameter in surveillance because any uncertainty about a positive result will quickly undermine participants' confidence in the program. Laboratory approaches to increasing diagnostic specificity (i.e by reducing false positives) include raising assay cutoffs, confirmatory testing of samples that screen positive, or re-sampling and re-testing procedures. Processes for dealing with unexpected positives should be established and agreed upon before initiating the program. Counterintuitively, diagnostic sensitivity is less important. While 'adequate' diagnostic sensitivity is necessary, detection does not have to be absolute because ongoing surveillance will reveal the true status of the population and lead to the agreed-upon response.

Although diagnostic specificity tends to be stable over time and across specimens, diagnostic sensitivity varies widely as a function of specimen and time post infection. For example, Table 3 shows that the choice of specimen has a major effect on the detection of early PRRSV infection in boars using RT-qPCR, with testing of semen samples producing many false negatives. The 'sampling effect' is even more profound in the case of chronic persistent

Table 3 Detection of early PRRSV infection in individual boars using RT-PCR by specimen and day post inoculation (Pepin et al., 2015)[a]

Specimen	Percent (%) positive by day post inoculation						
	1	2	3	4	5	6	7
Serum	36.5	79.1	89.5	93.8	95.2	97.4	99.9
Blood swab	30.3	73.3	79.4	86.7	87.9	99.9	99.9
Oral fluid	3.6	59.0	89.4	97.6	99.9	99.9	99.9
Whole semen or supernatant	0	12.8	17.1	26.5	22.0	28.9	30.8
Cell fraction semen	0	0	14.7	21.4	18.8	47.1	43.5

[a] Reprinted with the permission of John Wiley and Sons. Probability calculated from data available in published reports on the detection of PRRSV in boars under controlled settings and analyzed using a binomial logistic regression model with estimates obtained using the least square methods.

infections, for example, classical swine fever virus (Panyasing et al., 2018b), PRRSV (Henao-Diaz et al., 2020b), African swine fever virus (Carrillo et al., 1994) and latent persistent infections such pseudorabies virus (Beran et al., 1980). These pathogens often produce inapparent carriers, that is, animals in which the pathogen is present for weeks or months after their initial infection.

Using PRRSV as an example, viremia is observed beginning early in the infection. Resolution of viremia is not an indication of sterilizing immunity; rather the infectious virus remains in lymphoid tissues for an extended period. Thus, transmission of PRRSV to susceptible penmates was reported 99 days post inoculation, and isolation of PRRSV from tonsil-scraping samples for up to 157 days post inoculation (Wills et al., 1997; Zimmerman et al., 1992). Henao-Diaz et al. (2020b) estimated that 2% of the animals were viremic at 98 days post infection, but the infectious virus was present in 30% (Table 4). Thus, for chronic infections, a combination of antibody and RNA assays is typically required to achieve the highest rate of detection.

In the case of aggregate specimens, diagnostic sensitivity varies both as a function of the proportion of positive members in the pool and their stage of infection (Olsen et al., 2013). Logically, increasing the proportion of positive individuals contributing to the sample will produce a corresponding increase in the probability of detection, as shown for pen-based oral fluids (Table 5). Likewise, in Table 5, a comparison of oral fluid samples versus serum samples demonstrates the utility of aggregate samples in surveillance, that is, many more individual pig samples would need to be collected to match aggregate samples' probability of detection under the same circumstances.

Table 4 Predicted rate of PRRSV positivity over time in PRRSV-infected animals: serum RT-PCR, bioassay and serum antibody (Henao-Diaz et al., 2020b)[a]

Diagnostic approach[b]		Day post exposure to PRRSV or modified live vaccine							
	n	3	7	14	28	42	77	98	175
Detection of PRRSV RNA in serum	1973	94%	92%	87%	72%	48%	7%	2%	0%
Detection of infectious PRRSV[c]	468	ND	77%	74%	68%	61%	41%	30%	7%
PRRSV serum antibody[d]	1866	ND	ND	92%	92%	92%	91%	91%	90%

[a] Reprinted with the permission of Elsevier.
[b] Rates estimated based on prediction equations for PRRSV detection derived from aggregated data (*n* = 4307) from 19 refereed publications (1995–2018).
[c] Bioassay performed by inoculation of naïve pigs with lymphoid tissue homogenate or virus isolation in cell culture.
[d] Antibody detection in individual serum samples collected at ≥ 14 days post exposure using on IDEXX ELISA HerdChek® or IDEXX PRRS ELISA X3 Ab Test (IDEXX Laboratories, Inc., Westbrook, ME, USA).

Table 5 Effect of within-pen PRRSV prevalence on the probability of detecting RNA or antibody using one pen-based oral fluid sample. A comparison of the number of individual pig serum samples to achieve the oral fluid detection rate at the same prevalence (right half of Table 5).

Within-pen prevalence (%)	Probability of detecting PRRSV in a pen of pigs using one oral fluid sample (95% confidence interval)[a]		Number of pig serum samples needed to match the oral fluid probability of detection (95% confidence interval)[a]	
	PRRSV RNA	PRRSV antibody	PRRSV RNA	PRRSV antibody
5	0.31 (0.09, 0.67)	0.17 (0.06, 0.38)	8 (3, 17)	5 (2, 10)
10	0.79 (0.48, 0.94)	0.59 (0.37, 0.77)	11 (5, 16)	7 (4, 10)
15	0.94 (0.76, 0.99)	0.85 (0.67, 0.94)	12 (8, 16)	9 (6, 12)
20	0.98 (0.88, 1.00)	0.94 (0.82, 0.98)	13 (8, 16)	10 (7, 13)
25	0.99 (0.93, 1.00)	0.97 (0.90, 0.99)	13 (9, 16)	11 (8, 13)

[a] Detection of PRRSV RNA or antibody in pen-based oral fluids were analyzed by logistic regression to model the probability of a positive outcome by within-pen prevalence. The number of serum samples needed to match the probability of detection for one oral fluid sample was calculated from the probability of ≥ 1 positive test by prevalence and sample size assuming hypergeometric distribution (J. Zimmerman, personal communication).

6 Surveillance planning checklist and summary

Step 1 - Define the surveillance objective. Surveillance objective(s) should reflect the producer's goals and the conditions found in the production system. The more precise the question, the more likely the success of the surveillance program. The objectives should be completely clear to all involved. This is also the time to plan a response to a positive result, if a response is part of the program. In particular, unexpected positives are often highly disruptive to day-to-day operations. What is the action plan if an unexpected positive result is received?

Step 2 - Determine the sampling plan. Issues on sampling and testing are reviewed in Sections 4 and 5. Simplicity and consistency are key; a complex sampling plan either will not be carried out or will not be done correctly. For interpretable surveillance data, samples should be collected in the context of a population that shares a common environment (epidemiological unit), that is, the animals in a pen, room, or barn. Results from animals that do not share a direct epidemiological connection are often uninterpretable.

Determination of sample size is complicated by the complexity of farm populations. As discussed in Section 2, sample size calculations based on binomial sampling distributions assume that (1) the population is homogeneous, that is, randomly selected pigs in the population have an equal chance of being positive, and (2) the pigs in the population are 'independent', that is, the infectious disease status of one pig is not predictive of the status of another. These circumstances are only occasionally encountered on swine

farms. Nevertheless, if collecting individual discrete samples, for example, serum, the best option is to use binomial sample size calculations based on the population within each epidemiological unit.

Sample size calculators are not available for aggregate samples, for example, oral fluids. Rotolo et al. (2017) reported the probability of detecting PRRSV in wean-to-finish barns using oral fluids as a function of the number of positive pens. In the field, Ramirez et al. (2012) collected six pen-based oral fluid samples at two-week intervals in ten barns (most holding ~1 100 pigs) from placement to market. Testing by PCR detected porcine circovirus 2 and Torque teno sus viruses at one or more samplings in all ten barns, PRRSV in nine of ten barns, and influenza A virus in seven of ten. Thus, field results suggested that collecting ~6 oral fluids per 1000 growing animals provided a reasonable assurance of detection over time.

In the absence of broadly generalizable sample size calculations for pen-based samples, it may be useful to conduct a sample-size sensitivity analysis (Rotolo et al., 2017). Assuming independence among epidemiological units, that is, rooms or barns, the overall probability of detection from sampling ≥ 2 units is:

$$P = (1 - (1 - p_1)(1 - p_2)(1 - p_3)\ldots(1 - p_k)),$$

where p_i is the probability of detection in the ith (i = 1,2, …, k) unit. If the units are similar in design and are sampled using the same plan, then p_i can be assumed equal among units and the formula simplified to:

$$P = \left(1 - \left(1 - p\right)^k\right),$$

where

P = cumulative probability of detecting the target in ≥ 1 of the epidemiological units sampled,

p = $pr*pse$, where pr is the probability of selecting an infected pen within a unit for sampling and pse is the probability that the pen-based sample will test positive (see Table 5), and

k = number of units sampled.

The impact of the number of pen-level samples per unit on the probability of detection is embedded in 'pr.' That is, if we suppose that one of five pens is positive in each unit and the decision is to sample one pen per unit, then pr = 1/5 = 0.2. If there are five units, and the pen-level probability of detection is assumed to be 0.79 (this assumes within-pen prevalence of 10% - from Table 5), then the cumulative probability of detection among the five units is calculated as,

$$P = \left(1 - \left(1 - 0.2 * 0.79\right)^5\right) = 40.3\%.$$

If two pens per unit are sampled then $pr = 0.4$, and

$$P = \left(1 - \left(1 - 0.4 * 0.79\right)^5\right) = 68.0\%.$$

Sample size for breeding herds is more problematic. Typically, it is not practical to sample a statistically sufficient number of sows on a routine basis. Alternatively, oral fluid samples may be collected from group-housed sows, but behavioral and detection information is sparse (Pierdon et al., 2016). More frequently, the status of recently farrow litters or piglets within litters is used as a proxy for the sow herd itself using serum samples from suckling piglets, oral fluids, or processing fluids (Almeida et al., 2021a,b,c; López et al., 2021).

Although the information discussed above is all relevant to the question of sample size, the ultimate decider of sample size is the budget allocated to sampling.

Step 3: Sample allocation and frequency.

Infectious diseases in swine farms tend to cluster, that is, pigs physically close to each other are more likely to be of similar infection status versus pigs further apart. Conventional sampling strategies based on 'random' selection are usually less efficient and more expensive than spatially based methods because randomization tends to result in over- or under-sampling disease clusters. That is, the cluster is either not sampled (missed entirely) or sampled multiple times when a single sampling would suffice. In contrast, spatially based methods take spatial autocorrelation and heterogeneity into account. Thus, within rooms or barns, Rotolo et al. (2017) showed that 'fixed spatial' sampling was as good or better than random sampling and that repeatedly sampling the same pen(s) over time provided logical and interpretable data. Here, 'fixed spatial' sampling is defined as uniform spatial allocation such that samples are spread equidistantly to each other over the room or barn being sampled.

There are no formulas to determine sampling frequency. Ultimately, the driver is the urgency of detection (the more urgent, the greater the frequency). Based on the literature, one or two samplings per month provide interpretable and actionable data (Ramirez et al., 2012; Rotolo et al., 2017).

Step 4: Plan for data analysis.

Every surveillance program needs a plan for collecting, managing, and analyzing surveillance data. The particular analyses will depend on the specific objective(s). Regardless, statistical process control (SPC), an approach that originated from

quality control in industry, is highly useful in monitoring disease trends, detecting change points and understanding normal variation in the population. A description of SPC is beyond the scope of this review, but resources and examples of its use are widely available (Baum et al., 2005; de Vries and Reneau, 2010).

7 Conclusion

In swine production, herd immunity is manipulated to reduce clinical losses, biosecurity implemented to stop the entry of pathogens, and surveillance performed to verify that herd immunity and biosecurity are functioning effectively. Historically, surveillance was rarely feasible because of logistical and cost considerations, that is, sampling a meaningful number of individual animals was cumbersome, testing was slow and the entire process was expensive relative to the return. These impediments have largely been removed by developments in diagnostic medicine that provide for efficient sampling/testing and advancements in computer technology that facilitate the collection, manipulation and analysis of data. Importantly, these technical improvements are only tools whose use must be guided by a clear surveillance objective; a sampling/testing plan that is easy to implement, affordable and strategic; a system for the on-going analysis and interpretation of test data; and an action plan for responding to testing results.

8 Abbreviations

AB	Antibody
APPV	Atypical porcine pestivirus
ASFV	African swine fever virus
CSFV	Classical swine fever virus
DIVA	Differentiating infected from vaccinated
ELISA	Enzyme-linked immunosorbent assay
FMDV	Foot-and-mouth disease virus
HEV	Hepatitis E virus
IAV	Influenza A virus
LIMS	Laboratory information management system
NA	Nucleic acid, either DNA or RNA
OIE	World Organisation for Animal Health, formerly Office International des Epizooties (OIE)
PCMV	Porcine cytomegalovirus
PCR	Polymerase chain reaction
qPCR	Quantitative PCR (starting material DNA or cDNA)
RT-qPCR	Reverse-transcriptase qPCR (starting material RNA)
PRDC	Porcine respiratory disease complex
PCV2	Porcine circovirus type 2

PDCoV	Porcine deltacoronavirus
PEDV	Porcine epidemic diarrhea virus
PPV	Porcine parvovirus
PRRSV	Porcine reproductive and respiratory syndrome virus
PRV	Pseudorabies (Aujeszky's disease) virus
SVDV	Swine vesicular disease virus
SVV	Seneca valley virus
TGEV	Transmissible gastroenteritis virus
USDA	United States Department of Agriculture
VESV	Vesicular exanthema of swine virus
VSV	Vesicular stomatitis virus

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