

Addressing Swine Influenza A Virus

Guidelines for surveillance in pigs



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The European Swine Influenza Network (ESFLU) has been actively addressing the challenges posed by Swine Influenza A Virus (IAV-S) infection in pigs across Europe. Given the increasing circulation of swine influenza strains in European swine populations and the emergence of new variants, including those with zoonotic potential, strengthening surveillance and monitoring capabilities is crucial.

It is important to note that these guidelines provide a set of suggested methods which are not intended to endorse any specific company or commercial product.

Through collaborative efforts, the ESFLU network aims to harmonize analytical approaches, improve data sharing, and support coordinated responses to swine influenza, ensuring better protection of both animal health and public safety across Europe.

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Contents

Introduction	4
Background	5
1.1. Overview of circulating European subtypes and genotypes	5
1.2. Considerations	6
1.3. Clinical signs and evaluation of diagnostic test results	9
1.4. Rationale for the presented systems or scenarios	9
1. Diagnosis of influenza infection in pigs with respiratory signs	10
2. Monitoring influenza virus circulation at farm level	13
3. Serological surveillance across farms	15
4. Surveillance system to detect new strains, potentially with a zoonotic signature	17
5. Surveillance for freedom of Influenza virus infection	19
Definitions	21
Abbreviations	22
References	23
Appendix	24
1. Diagnosis of influenza infection in pigs with respiratory signs	24

Introduction

Swine Influenza A virus (swIAV) is both a major pathogen causing health and welfare problems in pigs globally, and an important zoonotic pathogen.

Since the virus (H1N1) that caused the Spanish flu in 1918 was detected in pigs, swIAV has been seen as a major concern regarding seeding a virus into humans that could spark a new influenza pandemic (Reid et al., 1999). In 2009, this concern became real, as a novel pandemic in humans was due to a reassortant Mexican swine H1N1 virus (H1N1pdm09) carrying genes both related to the Spanish flu, an American swine H3N2 virus and a European swine virus (Eurasian avian-like swine H1N1) (Mena et al., 2016). Following the 2009 pandemic in humans, the virus was re-introduced into the global pig population.

To enable timely detection and reaction upon change of influenza viruses or their impact in pigs, adequate surveillance systems should be in place. Surveillance of swIAV, for example, should include national surveillance activities that collect samples, perform initial tests and ideally perform basic genetic characterization of the identified viruses. Ideally, national surveillance activities for swIAV are coordinated and collected on a European level, ensuring timely, representative and cost-effective actions. ESFLU is a multi-stakeholder COST Action network of European scientists and veterinarians that focuses on research and surveillance of influenza A virus infections in pigs. ESFLU has developed multiple guiding documents to aid the veterinary field regarding sampling and diagnosis of influenza virus infections in pigs. Please consult the ESFLU website¹ for more information.

Instructions and disclaimer

This guideline aims to support veterinarians, scientists and policy makers to develop and execute meaningful influenza surveillance on either herd, regional or national levels. Although the developers of this guideline have aimed to develop broadly applicable guidelines, there will be needs to develop local, context-specific adaptations. For instance, if regional differences imply higher or lower general risks for infection or detection, one must account for this in the assumptions underlying which herds to sample or how large sample sizes need to be. In addition, in some local contexts, farm or herd characteristics may imply a different risk for which one has to account for. In other words, this guide is merely aiding in decision-making for the design and implementation of efficacious surveillance of influenza virus infections in pigs.

¹ www.swineflu.eu

Background

Influenza A viruses (IAV) have a negative-sense RNA genome that is divided into eight segments. Each segment encodes a specific protein of the virus. In practice, the most renowned are the hemagglutinin and neuraminidase proteins, encoded by the HA and NA segments. The combination of these two proteins determines the subtype of Influenza A virus. IAV that circulates in swine are defined as swine influenza A virus (swIAV).

The segmented RNA makes IAVs capable of evolving through two different mechanisms: viral drift and viral reassortment (Forrest & Webster, 2010). Viral drift is caused by point mutations due to IAV being an RNA virus that does not have a proofreading capacity when generating novel genomic RNA for virus progeny. Viral reassortment, or shift, can occur when viral segments of different IAVs are mixed during co-infections of a host. Novel gene segment combinations will give rise to novel genotypes, and sometimes novel subtypes if a new combination of the HA and NA surface genes is generated (Kim et al., 2018). All influenza pandemics since the Spanish Flu have been caused by a reassortant IAV. Although reassortment is considered to occur rarely, genetic drift and shift may have a major impact on the efficacy of a given vaccine, may change the viral dynamics in the herd and negatively impact the sensitivity of the diagnostic assays. In practice, the use of subtype designation is still widely applied, but it is important to consider its limitations.

Overview of circulating European subtypes and genotypes

Overall, four different subtypes are present in the pig population - H1N1, H1N2, H3N2 and more rarely, H3N1. H1Nx subtypes can be further classified into different lineages based on the origin of the HA gene segment, for example avian (1C), human (1B) or pandemic H1N1pdm09 (1A) origin, and in this guideline, the different combinations of surface genes of different lineages are referred to as HA and NA combinations (Anderson et al., 2016). When the lineages of all eight gene segments are available, the genotype can be determined based on the origin of each gene segment.

To help the reader, a short introduction to the nomenclature of the circulating swIAVs is presented in the following section, in addition to a historical background for each swIAV introduction.

There is a huge variety of subtypes and genotypes circulating in European pigs; however, the most prevalent subtypes in Europe are:

H1avN1av/Eurasian avian-like swine H1N1 (1C.2 viruses): This virus was introduced as a whole virus from birds to pigs in Europe at the end of the seventies or beginning of the eighties, and it quickly became enzootic and still constitutes one of the dominating subtypes.

H3swN2sw/swine-adapted Hong Kong H3N2: This virus originated from the human pandemic initiating in Hong Kong in 1968. Following the human pandemic, it adapted to swine, and in 1984, it reassorted with the above-mentioned Eurasian avian-like swine H1N1, where it retained its surface genes but gained an avian-like internal gene cassette. This virus still circulates in some but not all European countries

H1avN2sw (1C.2): This virus has the H1 of Eurasian avian-like swine origin and the N2 of the H3swN2sw virus. The initially documented H1avN2sw had the internal gene cassette of Eurasian avian-like origin and probably evolved in Denmark around 2003. The virus has since spread to several European countries. After 2009, the virus reassorted with the H1N1pdm09 virus (1A3.3.2) to generate several different internal gene cassettes, with a full internal gene cassette of H1N1pdm09 origin being the most prevalent.

H1N1pdm09/H1pdm09N1pdm09 (1A.3.3.2): This virus caused the human influenza pandemic in

2009 and originates from Mexican swine. The HA, NA, and the internal genes are different from the other enzootic subtypes, and this virus is also considered enzootic in most European countries.

H1huN2sw (1B): This virus was first detected in England in 1994 and is a reassortant carrying an HA gene of the human seasonal flu and the NA gene of H3swN2sw origin. This virus circulates in the majority, but not all European countries.

Internal gene cassette: To describe the origin of the six gene segments (PB2, PB1, PA, NP, M, NS) making out the internal genes of the swIAV genotypes the abbreviation "P" is used for H1N1pdm09 origin (1A.3.3.2) and "A" for Eurasian avian-like H1N1 (1C) origin, and one letter for each gene, so that "PPPPPP" for example indicate a complete internal gene cassette of H1N1pdm09 origin.

Considerations

Clinical considerations

SwIAV mainly causes respiratory disease as the virus targets the epithelial cells of the respiratory tract such as the nasal, tracheal and bronchial epithelium causing destruction of the epithelium and mucosa and interstitial pneumonia mainly affecting the cranial and apical lung lobes. The clinical signs observed constitute signs of respiratory disease similar to those observed in humans infected with influenza A virus (IAV) that include fever, lethargy, loss of appetite, nasal secretions, coughing, and sneezing (Brown et al., 1993; De Vleeschauwer et al., 2009). As swIAV destroys the epithelium and alters the cytokine response, swIAV-infected pigs can be more susceptible to other viral or bacterial infections causing severe pneumonia. Co-infections can lead to exacerbated disease and higher mortality than expected for an infection with a single pathogen (Opriessnig et al., 2011). Therefore, swIAV is also a significant factor for antibiotic usage in pig herds. The clinical and epidemiological situation and the diagnostic objectives are relevant for decision-making regarding the specific animals to sample, sample types and sample storage and subsequent processing.

We refer to the ESFLU SOP for guidance on personal safety and proper sample handling, storage and transport conditions².

Sample type considerations

Different sample types can be used for the detection of evidence of swIAV infection in pigs (Table 1). Traditionally, nasal swabs (NS) have been the sample of choice for detection of swIAV in live pigs by PCR and from tissues from dead pigs (Slomka et al., 2010). Next, there is an increasing use of oral fluid for detection of swIAV, similar to other respiratory viruses. Oral fluid is collected either by the use of ropes or swabs for detection on group or individual level. In addition, udder wipes (UW) can be used to indirectly collect both nasal secretions and saliva from suckling piglets. Both udder wipes and oral fluid collected by rope are at risk of being contaminated from the environment. Although increasing evidence becomes available that oral fluids and udder wipes are suitable for detection of swIAV by PCR (Stadler et al., 2024, Garrido-Mantilla et al., 2019, de Lara et al., 2022), for virus isolation or genotyping by sequencing may require individual samples where throat samples followed by NS and nose wipes (NW) may be most suitable (Garrido-Mantilla et al., 2019). However, higher detection rates were demonstrated for group samples and environmental samples compared to individual samples, except for throat swabs (Garrido-Mantilla et al.,

² [ESFLU Guidance: Laboratory Protocols for the Detection and Monitoring of Swine Influenza A Virus](#)

2019). Moreover, pen-side tests (PST) for swIAV exist, but these are often developed for human use. Their performance may also vary depending on sample type, vaccination status and time of sampling in relation to infection (Goodell et al., 2016).

As an example, in a study testing two RT-qPCRs, high sensitivity (> 93%) and specificity (> 95%) were found for both respiratory swabs and tissues from pigs infected with H1N1 (Slomka et al., 2010). In addition, a wide portfolio of sample materials was tested in one study, including NS, throat swabs, oral fluid samples, surface wipes, udder wipes, airborne particles and air samples (Garrido-Mantilla et al., 2019). All sample materials came from either piglets or nursery pigs and were tested by RT-qPCR. Group samples and environmental samples had a higher odds ratio of being positive by RT-qPCR than individual samples, except for throat swabs. In terms of virus isolation, individual samples performed best, and from nursery pigs, the success was greatest with throat samples followed by NS and nose wipes (NW), where only the external part of the snout is sampled.

Pen-side tests (PST) for swine influenza virus exist, but these are often developed for human use. In a controlled study, a pen-side test detected swIAV in pigs during the first five days after infection with a positive predictive value of 100% (no false positives). However, the diagnostic sensitivity for oral fluids samples on days 1–5 was only 51.3%, with higher sensitivity in unvaccinated pigs (64.6%) than in vaccinated pigs (20.6%) (Goodell et al., 2016). In addition, the study found that virus isolation was more probable from NSs than oral fluids, but, like PST, less successful after day 5. The time of infection for pigs on farms is not known and therefore when testing subacutely affected pigs, diagnostic tests may return negative. Routine analysis of environmental or dust samples for detection and genotyping is still fairly uncommon because of low success rates, but this field is continuously developing.

Serology can be used for surveillance at the herd level to measure influenza status, however, serology is not recommended for rapid clinical diagnosis. Moreover, due to high level of cross-reactivity among circulating subtypes, it may prove difficult to identify subtype based on serology. Paired blood samples can be of value to associate the increase in antibody levels in diseased pigs of approximately 12 weeks of age and older to the presence of clinical signs. However, interpretation may be hindered in younger pigs as pigs that get infected in the presence of maternally derived antibodies frequently do not develop a detectable antibody response.

Table 1. Summary of the most used samples materials

Sample material	Advantage	Comment
Nasal or pharyngeal swabs	Sensitive and easy to collect from affected pigs	Can be pooled for subsequent analysis
Nose wipe	Non-invasive, easy collection	Risk of environmental contamination
Oral fluid (by swab or rope)	Sample (larger) groups of pigs with less effort and no discomfort. Sensitive for detection of IAV by PCR	Easily contaminated by feces or feed. WGS may not provide reliable detection of variants due to contamination or by the presence of multiple viral strains in one sample.
Tracheobronchial swab (TBS) /BALF (ante mortem)	Very sensitive, valuable for subsequent WGS	Requires sedation of pigs. Sampling and restraint can cause discomfort. These disadvantages outweigh the possible benefits.

Udder swab (or wipe)	Non-invasive	Aids indirect detection of swIAV in suckling piglets. Contamination possible by organic material. WGS more likely unsuccessful due to presence of multiple strains or contamination.
Serum/plasma	Detection of antibodies	Antibodies are detectable from ~10 days post infection. PCR tests usually test negative on serum as viremia is unlikely.
Tracheobronchial/bronchial swab or lavage (<i>post mortem</i>)	Very sensitive, valuable for subsequent WGS	Requires sacrificing characteristically affected individual pigs. Pigs found dead may not have suffered from swIAV. Can be performed at least 24h after death if carcass was cooled and transported adequately.
Lung tissue (<i>post mortem</i>)	Sample deviant tissue or specific tissue	Requires sacrificing characteristically affected individual pigs. Pigs found dead may not have suffered from swIAV.
Air	Non sensitive. Positive result, possibly indicative of airborne transmission	Requires advanced air collection methods. Sequencing frequently unsuccessful and detecting infectious virus very challenging.
Dust wipe	Sensitive to detecting swIAV RNA	Not routinely feasible for subsequent virus characterization except by specific PCR tests, Not feasible to isolate virus

For more detailed information about laboratory tests, the reader is referred to the ESFLU Laboratory Guideline³.

³ [ESFLU Guidance: Laboratory Protocols for the Detection and Monitoring of Swine Influenza A Virus](#)

Clinical signs and evaluation of diagnostic test results

It is important to take into consideration that any diagnostic test will produce a result that may or may not be relevant to the question at hand. It is up to the veterinarian to select the appropriate test system, diagnostic test and to interpret the outcomes.

Each diagnostic test, such as an antibody or PCR test, can be characterized by the test sensitivity and specificity. As such, the clinical assessment by a veterinarian can also be considered a 'diagnostic test'. The sensitivity represents the proportion of true positive cases that test positive by a diagnostic test, and the specificity represents the proportion of true negative cases that test negative.

Next to the test characteristics, the probability of a test outcome in each situation will also rely on the true prevalence in that situation. In a disease-free situation, a positive test result is not as probable as a negative one. In case of frequently occurring endemic disease, a positive test result is more probable than a negative test result.

Selection of animals to sample may depend on clinical signs observed in the affected animals, their pen mates, or animals from the same production category. In young pigs (<4 weeks), infections may also run a subclinical course and later be complicated by secondary infections. In affected piglets, samples for PCR testing may test positive only shortly before (~ 1 day) and after ($\sim <5$ days) observing clinical signs, after which the probability of positive tests decreases. After infection, antibody levels will increase in an infected pig. One can use paired serology to detect a significant rise in antibody levels by testing the same animal twice with an interval of, at least, 10 days. Since preexisting antibodies may interfere with the development of antibodies, paired serum samples should only be used in naive pigs.

For the appreciation of the diagnostic test outcome, one can therefore use a set of different parameters, e.g. the positive and negative predictive value, the likelihood or odds of the outcome. For specific example scenarios in the next chapters, these assessments will be discussed.

Rationale for the presented test systems/scenarios

This guideline aims to aid veterinarians or farm advisors and farmers, as well as policy makers, in answering specific questions regarding swIAV infections in pigs.

1. [Diagnosis of Influenza infection in pigs with clinical signs](#)
2. [Monitoring influenza virus circulation at farm level](#)
3. [Serological surveillance of swine influenza across farms](#)
4. [Surveillance system to detect new strains, potentially with a zoonotic signature](#)
5. [Surveillance for freedom of influenza virus infection](#)

In general, with an animal health surveillance system, one aims to collect animal health data to subsequently take an informed, pre-defined decision for action. In cases where no action is taken, one usually is talking about monitoring. Animal health monitoring may feed decision-making in the long run, but actions are usually less predefined. These scenarios in general cover most objectives for diagnostics, monitoring and surveillance of swIAV virus in pigs. The design of sample strategies should be adapted to local context and needs to be based on prior assumptions on expected prevalence, knowledge of circulating strains and specific exposure risks. Please consult local experts to ascertain a valid approach and agreement on the interpretation of results.

1. Diagnosis of influenza infection in pigs with respiratory signs

This specific guideline serves a twofold purpose.

1) It aids the veterinarian and farmer to establish whether there is an association between clinically diseased pigs (on farm level) and the presence and identification of swine influenza virus, and thus helps to make a diagnosis and

2) the outcomes may feed surveillance on an aggregated level (see strain surveillance). One of the components in that system will refer to this guideline.

Aim/purpose	Diagnosis of influenza A virus as the likely causal pathogen for diseased pigs with signs of respiratory disease ⁴
Background	Influenza can cause respiratory disease and fever in pigs, characterized by fever, lethargy, loss of appetite, nasal secretions, coughing, and sneezing. In young pigs (<4 weeks), infections may also run a subclinical course and later be complicated by secondary infections.
Level	Herd level
Sample type	NS, OF, UW, tissue for PCR testing Serum for paired serology
# Samples	5 pigs: with characteristic clinical signs or in a known high-prevalence setting. 10 pigs: in case of expected low or unknown prevalence (Figure 1).
Animal	Pigs with acute signs of respiratory disease (preferably within 48-72 hours after onset); fever, lethargy, loss of appetite, serous nasal secretions, coughing, and or sneezing. Preferably sample pigs from multiple litters/pens within 48 – 72 hours after onset of clinical signs. For antibody testing, the same ~10 animals are tested twice with a minimum span of 10 days (preferably 14 days) (paired serology).
Frequency	To set a diagnosis for a first episode of disease, one-time sampling can be sufficient. On herd level, repeated episodes of influenza illness are not necessarily caused by the same influenza strain. For that reason, sampling should be repeated at least annually.

⁴ This protocol does not cover the evaluation of other potential pathogens as cause of respiratory disease. Please consult your veterinarian or local animal health service.

If the results are inconsistent with clinical signs or the disease is not resolved, one should redo the diagnostic process as another cause of disease cannot be excluded.

Lab analysis	Virus detection by RT-PCR. Samples from up to 5 animals can be pooled if needed. Subtyping by subtype-specific RT-PCR or more detailed typing by WGS. Samples with higher viral loads have a higher probability of successful subsequent analyses such as genotyping.
Interpretation	PCR positive results with low Ct values ($< \sim 33$) may lead to the conclusion of influenza virus infection in these pigs. Ct values < 25 are indicative of high viral loads present. The interpretation of test results should be considered given the prior assumption of likelihood of influenza infection. A positive test result is highly likely to reflect infection in a high probability scenario, whereas a negative test result is not (See Note and Appendix) In case > 6 of 10 animals develop a significant rise in antibody levels in a paired serology test, the probability of infection by influenza is high. In a test this could be reflected by a > 4 times increase in antibody titres (so-called seroconversion).
Limitations	Proper clinical diagnostic skills needed to identify and select representatively affected pigs. Detection of swIAV is limited to a short time frame, as many pigs only test positive for 3-5 days post-infection. Sampling of subacutely affected pigs should be avoided as in these pigs the virus may not be detectable and subsequent bacterial infections may be detected. Paired serology can be complicated in younger animals, given the presence of maternal antibodies. Also, some antibody tests have lower sensitivity and specificity if the antigens of tests differ from the test antigen .
Benefits	Limited number of samples required. Basic laboratory tests required for PCR and serology testing. The outcome can serve (zoonotic) strain surveillance objectives, and as such, this serves as a passive surveillance system.
Who	Veterinarian together with farmer/caretaker

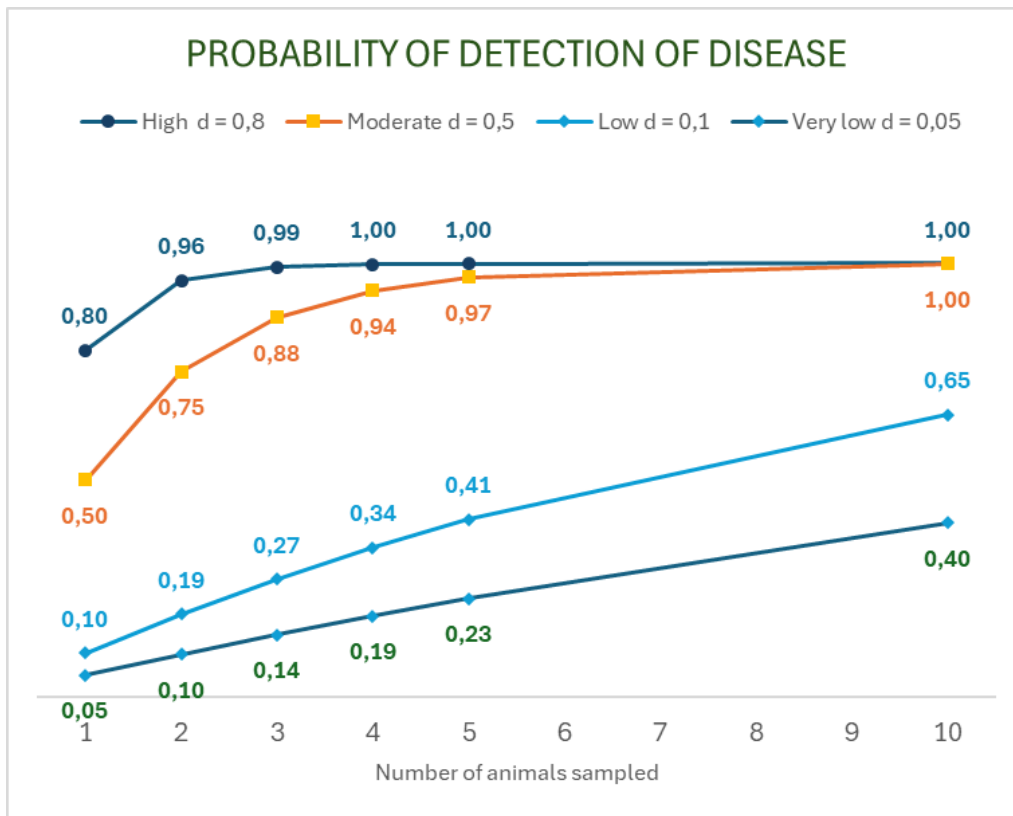


Figure 1: Calculated probability of detection of infection given the number of animals sampled (x-axis) and the expected pre-test prevalence (d). In other words, when you are quite confident that the pathogen is present with a high prevalence, only a few animals need to be sampled to have a high probability of detection. If the expected prevalence is low, one needs to sample more pigs to detect the infection. Important to note is that one positive test result will imply that the pathogen is present, irrespective of the number of positive test results.

2. Monitoring influenza virus circulation at farm level

Aim/purpose	To detect influenza A infection in pigs on farm level (irrespective of clinical signs)
Background	Influenza A infections in pigs can be present without inflicting clear clinical signs. Information about whether influenza is circulating in the herd (or herd sublevels) and which influenza subtype or strain may be relevant to inform pig farmers, traders, personnel, veterinary and/or public health authorities. It is known that more than one influenza strain can circulate on a farm. Therefore, subsequent testing for virus characterization should be considered for samples from more than one age group depending on the specific herd structure. Prioritising which samples to choose for subsequent analysis can be done on presumed viral load (by qPCR Ct value), by presence of clinical problems, or based on knowledge from previous monitoring attempts.
Level	Farm level
Sample type	OF, NS, UW
# Samples	The sampling strategy depends on the expected prevalence within the farm and the age group targeted. 5 pigs per age group; in case of moderate prevalence (0.5) -> probability of detection is 0.97 (See Annex 1, table A1). 10 pigs per age group; in case of moderate to low prevalence (0.25) -> probability of detection >0.9. Based on the literature, the expected prevalence in some herds suffering from influenza virus infections can be high in suckling pigs and weaned pigs. Therefore, using 10 samples generally provides a high probability of finding influenza in the age group. >20 pigs per age group; in case of low prevalence (<0.1) -> to reach a probability of detection >0.9. In case larger groups of animals are to be sampled, a combination of OF from weaned pigs and NS samples from suckling pigs can be considered.
Animal	Suckling pigs, weaned pigs, finishing pigs, gilts, sows Without prior information, a cross-sectional design of 10 suckling pigs and 10 weaner pigs is a good starting point. For random sampling, it is important to sample a maximum of one pig per pen.
Frequency	Once

Lab analysis	PCR for influenza A with subsequent subtype or strain typing Samples from up to 5 age-matched animals can be pooled if needed. Pooling samples from animals from different age categories is not recommended.
Interpretation	The positive predictive value is high for any prevalence assumption > 0.1. The negative predictive value is low for any assumed prior prevalence > 0.6. (assuming test specificity of 0.99). Different swIAV types may circulate in different animal categories, therefore limited downstream analysis can create selection bias or fail to detect all circulating swIAV types in the farm. Repeated analysis over time (e.g. annual) is recommended to detect circulation of different types of swIAV.
Limitations	The number of samples does not suffice to monitor trends of within-farm prevalence over time.
Benefits	Acceptable cost/benefit ratio given the number of samples and possibility to pool.
Who	Farmer and/or vet

3. Serological surveillance across farms

Aim/purpose	To obtain a cross-sectional overview of influenza A virus circulating in pigs in a predefined geographical area and period
Background	Influenza A infections in sero-negative pigs will induce an antibody response.
Level	Across farms on a geographical (e.g. national or regional) level in a specific period (e.g. one year)
Sample type	Blood (serum)
# samples	The number of samples depends on: - the expected prevalence across farms - the expected prevalence within farms - the desired precision of the prevalence estimates - its variation over time (for instance seasonal).

Multi-stage sampling calculations can be performed to define the number of animals to sample.

In general, one can assume to sample ~385 farms to detect an apparent prevalence of 0.5 and estimate it with a precision of 0.05 (meaning from 0.45-0.55). The sample size is larger for estimating an apparent prevalence around 0.5 than lower (e.g. 0.2) or higher (e.g. 0.8).

The number of samples within a farm depends on the expected within-farm prevalence in a similar way (see Table 2 for examples).

Animal	Pigs (e.g. pigs at slaughter).
Frequency	Once; samples plausibly being distributed over a defined period.
Lab analysis	Serological test; e.g. Pan-Influenza A ELISA or HI
Interpretation	A positive test implies that the pig has previously been infected with swIAV or has received maternal derived antibodies from the sow. False negative results cannot be excluded in case the pig was infected in the presence of high levels of maternal antibodies, as such pigs may not seroconvert upon infection.
Limitations	General influenza A virus antibody response can be detected using broad-reacting serological tests such as an NP-based ELISA. Subtype-specific antibody responses can be measured using specific diagnostic tests, such as HI or Luminex, but the sensitivity and specificity is highly dependent on the strain used as antigen.

Young piglets may test positive, due to presence of maternal antibodies and these results should be considered with caution.

Serological assays have several disadvantages as both sensitivity and specificity may be lower in case the assay-strains are not updated to the circulating strains. Consequently, both false negative reactions and false positive reactions against related antigens, due to cross-reaction, may occur.

Benefits	Sampling can be done at the slaughterhouse or can be obtained from samples taken for other purposes. Serum can be stored until analysis.
	Serological assays are less costly.
Who	Veterinarian or slaughterhouse in collaboration with the laboratory.

Table 2 Sample size

Example of a sample size calculation to detect infection in a herd and estimation of the percentage of farms affected, with 95% confidence (total # samples; total # of herds/# samples per herd per time period). The absolute precision was set at 1%, 2%, 3%, and 5% for a between-farm prevalence estimate of 1%, 5%, 10%, and $\geq 25\%$, respectively; the farm size was set to 1,600 fattening pigs.

In bold, the number of farms to sample when the expected within farm prevalence is 50% and the between farm prevalence estimate is expected to be estimated with 5% precision. Based on Germeraad, Bouwknegt et al., (manuscript in preparation) and assume perfect test characteristics.

		Expected prevalence within farms				
Estimate (precision)		1%	5%	10%	25%	50%
Between-farm prevalence	1% ($\pm 1\%$)	88,773	21,336	11,049	4,191	1,905
		381 / 233	381 / 56	381 / 29	381 / 11	381 / 5
	5% ($\pm 2\%$)	106,481	25,592	13,253	5,027	2,285
		457 / 233	457 / 56	457 / 29	457 / 11	457 / 5
	10% ($\pm 3\%$)	89,705	21,560	11,165	4,235	1,925
		385 / 233	385 / 56	385 / 29	385 / 11	385 / 5
	25% ($\pm 5\%$)	67,337	16,184	8,381	3,179	1,445
		289 / 233	289 / 56	289 / 29	289 / 11	289 / 5
	50% ($\pm 5\%$)	89,705	21,560	11,165	4,235	1,925
		385 / 233	385 / 56	385 / 29	385 / 11	385 / 5

4. Surveillance system to detect new strains, potentially with a zoonotic potential

Aim/purpose	Detection of new strains with a zoonotic potential by studying the genetic make-up of strains circulating in a population.
Background	For avian influenza viruses several genetic markers for increased zoonotic potential have been described, however this is not the case for swine influenza viruses (Anker et al., 2025). Thus, there is not a fixed and documented protocol for the surveillance of increased zoonotic potential of viruses detected in swine (Coggon et al., 2025). The first step in a zoonotic analysis is whole genome sequencing (WGS). The sequences are analysed for the presence of gene segments of human seasonal or avian origin, mutations leading to development of antiviral resistance, mutations that impact recognition of antiviral cellular factors, innate immune escape mechanism and mutations in antigenic sites. A surveillance system for the detection of zoonotic signatures could be based on multiple components, depending on the local context meeting different farming or housing systems with different risk levels (e.g. extensive smallholder outdoor pig farms and commercial large scale pig farms with endemic swIAV circulation). A zoonotic variant in pigs is not necessarily pathogenic for pigs. Still, it is recommended to sample clinically affected pigs (if present) to avoid too extensive sampling. This should be taken into consideration when interpreting the data, because the system is not sensitive to detect subclinical circulating virus strains.
Sample type	NS, OF, TS
# samples	Based on limited experiences, a rough estimate could be provided that in case >80 sequences are determined at the regional/national level during a given period, all patterns in circulating variants will have been detected⁵. However, this estimate can be different, based on country and pig industry characteristics. The previously given guidelines 1 and 2 can be used to determine the sample size and which animals to be sampled on farms.
Animal	See guideline 1 and 2.
Frequency	Once per period. Annually is recommended as a minimum reporting frequency.

⁵ Based on Meijer et al., Report on Swine Influenza Surveillance 2024-2025, the Netherlands, <https://dx.doi.org/10.21945/RIVM-2025-0150>

Lab analysis	PCR with subsequent genetic analysis of parts or the full influenza genome.
Risk strata	Assuming no prior information on exposure risk, one must assume equal risk for different farms and different animal categories within farms. In case specific risks can be attributed to, for instance outdoor farms in a high-risk period of influenza virus circulation in wildlife, one can aim to include a higher proportion of such farms in the surveillance program.
Limitations	As there is no common definition of a 'potential zoonotic pandemic' strain and the exact characteristics to search for are unknown, the approach is unspecific by exploring the variation of circulating viruses. One cannot define a specific lower limit prevalence nor provide a probability of freedom from specific infections.
Interpretation	Identification of viruses with new gene constellations should be flagged, especially if the virus contains gene segments from humans or non-porcine animal species and/or has critical mutations (mutations known to lead to antigen escape, antiviral resistance, change in receptor affinity etc.). Subsequent analyses could include virus culture and phenotyping of the viral strains. Results should be communicated to the national influenza reference laboratory, that in turn will inform the relevant authorities. Ideally, rapid communication of results to a wider European surveillance network is performed to enforce surveillance actions or enable a coordinated response.
Who	Veterinary authorities in collaboration with public health institutes, veterinarians and farmers

5. Surveillance for freedom of influenza virus infection

Aim/purpose	1. To document freedom from infection with influenza virus A in pigs in a defined geographical area or at the individual herd level. 2. To prove freedom from infection for specific HA types of influenza virus (e.g. H5 AIVs).
Background	Specific countries, regions or farms may be free from influenza A virus infection, or specific types of influenza virus. A need for proof of freedom from infection for a specific influenza type may be requested from veterinary or public health authorities or producer organisations in relation to trade.
Sample type	1. NS, OF, TS, UW, serum 2. NS, OF, TS, UW
# samples	The number of samples is defined based on the design prevalence level within and between farms. Although corrections for farm size can be applied in smaller farms, one generally can assume an unknown farm size due to the large number of animals present. The number of farms to be sampled can be marginally lower if the number of farms present in the population is only below 400. In case both a target between-farm prevalence of 0.05 and a within-farm prevalence of 0.05 is used, 65 animals should be sampled per farm from 62 farms (Table 3). Serological assays are not recommended for purpose 2 as cross-reaction between antibodies to different subtypes can occur. An exception may be to use serological assays for antibodies to other influenza A viruses (e.g. H5 or H7) than the swine influenza virus.
Animal	Pigs
Frequency	Cross-sectional, once
Lab analysis	Purpose 1. Serology or PCR Purpose 2. PCR + subtype PCR and/or WGS Proper confirmatory tests are required, in case of positive reactions.
Risk strata	Based on the expected higher probability of infection in weaned pigs, it is best to sample the end of the weaner stage if PCR tests are used or the end of the finisher stage for serology. Based on the same assumption, one can choose to have a higher proportion of breeding/multiplier farms included or farms that have had higher risk of exposure due to introduction based on expert opinion.

Interpretation In a population assumed free from infection, there is a low probability of positive results and as such, positive results need to be confirmed. False positive results can occur between different influenza strains when using serological assays (see scenario 3).

False negative results may occur in case animals are sampled between infection and development of immunity.

In a population free from influenza, pigs are expected to have no prior specific immunity against influenza A and as such, infection is more likely to inflict clinical signs. Therefore, random sampling can be partially replaced by targeted sampling of clinically affected pigs to increase the probability of detection.

Who Animal health program designers, pig integrators or authorities

Table 3. Example of sample size calculation for surveillance of freedom from infection (total # of herds to sample/# samples per herd). The farm size was set to unknown. In bold, the number of farms to sample when the target prevalence on farm level and within farms is set at 5%. Calculations were made using Epitools⁶ with assumed test sensitivity of 0.93 and target farm level and system level sensitivity of 0.95.

		Target prevalence within farms			
		2%	5%	10%	20%
Target between-farm prevalence	2%	157 / 160	157 / 65	157 / 33	157 / 17
	5%	62 / 160	62 / 62	62 / 33	62 / 17
	10%	31 / 160	31 / 65	31 / 33	31 / 17
	20%	15 / 160	15 / 65	15 / 33	15 / 17

⁶ <https://epitools.ausvet.com.au/twostagefreedom>

Definitions

Monitoring	Monitoring is the term describing a continuous effort to collect data to detect changes or trends in the occurrence, in this case, influenza virus infection in pigs, to inform decision-making.
Surveillance	Surveillance is a special case of monitoring where the ongoing collection, validation, analysis, and interpretation of health and disease data are used by key stakeholders to plan and implement more effective, evidence-based (public health) policies and strategies relevant to the prevention and control of disease.
Surveillance component	A specific part of a surveillance system that contributes to the higher objective, but may need to be addressed specifically due to specific risk factors such as animal species, sub-population, farming method, infection status, etc. Several components could be distinguished that may contribute together to a general objective.
Passive surveillance	The data collector awaits submission of disease data or test results. For instance, for case reporting.
Active surveillance	The data collector is actively searching, surveying, or testing animals to obtain relevant disease data or test results.
Case	A case is an animal or unit that fulfils the specific definition based on clinical, laboratory, or epidemiological characteristics. For this document, cases all consider pigs infected with influenza A virus, clinically or subclinically, defined by different laboratory tests.
Influenza virus strain	An influenza virus strain is a well-characterized isolate of influenza A virus
Influenza virus variant	The term variant is often used to describe a strain that is deviant from another, which could be on genotype, subtype or clade level. In human medicine, a variant influenza strain is used to describe any animal-derived strain detected in humans, and therefore, care should be taken when this term is used.
Influenza virus clade	A clade is a cluster of more 'alike' virus strains based on their genetic characteristics of the HA gene. For swine, H1N1 viruses are divided into A (classical swine), B (human like) and C linkage (avian-like).
Influenza virus genotype	Influenza A virus genotypes are defined based on their complete genome, designated by letters and numbers to reflect their specific genetic constellation and common ancestry.
Influenza virus subtype	Influenza A virus subtypes are defined based on different combinations of the hemagglutinin (H) and neuraminidase (N) proteins present.

Abbreviations

BALF	Broncho-Alveolar Lavage Fluid
GS	Genetic sequencing
HI	Hemagglutination Inhibition Test
IAV	Influenza A virus
NS	Nasal swab
NW	Nose wipe (external part of the nose)
OF	Oral fluid (collected by swab or rope)
PST	Pen - side test
PRRSv	Porcine Respiratory and Reproductive Syndrome virus
TS	Tissue sample of lower respiratory tract (trachea, lung parenchyma, etc).
UW	Udder wipe
#	number

References

Links to Webpages

[A Phylogeny-Based Global Nomenclature System and Automated Annotation Tool for H1 Hemagglutinin Genes from Swine Influenza A Viruses. | J. Craig Venter Institute](#)

[Evaluation of sampling methods for effective detection of infected pig farms during a disease outbreak - PMC](#)

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Appendix

1. Diagnosis of influenza infection in pigs with respiratory signs

Scenario with pigs with similar characteristic clinical signs

The likelihood of disease can be determined when combining the test results with the expected prior probability of infection. For instance, the veterinarian may, based on historical herd information or by experience, have a higher or lower prior probability of finding influenza in the sampled pigs. If one combines this prior information with test characteristic estimates, the odds of disease, given a test result, can be calculated. The odds of disease, when a positive test result is obtained, strongly decrease when the prior probability of disease is lower (Table 4).

One can assume a higher prior probability of infection in case, for instance, a high proportion of pigs of a certain age show similar clinical signs, characteristic of influenza infection, including dyspnea and fever. One should assume a lower prior probability of infection if only a few pigs show non-specific clinical signs. Probability estimates can range between 0 and 1. In case clinical examination on group and individual level, 3 – 5 individuals with characteristic clinical signs for influenza A virus infection can be identified, one can assume the following for the herd using a diagnostic test with a high-test sensitivity ($Se = 0.93$) and test specificity ($Sp = 0.99$) in four different scenarios with different pre-test probabilities of infection.

Table 4 Likelihood and probability of infection, in four scenarios. Table constructed using Epitools⁷, using assumed diagnostic test characteristics ($Se = 0.93$ & $Sp = 0.99$).

Input	Agreement with clinical signs for IAV			
	High	Moderate	Low	Very Low
Pre-test probability of infection	0.8	0.5	0.1	0.05
Output				
Post-test odds of infection in case of positive test result	372.00	93.000	10.300	4.890
Post-test probability of disease (PPV & 1 - NPV) for positive test result	0.997	0.989	0.912	0.830
Post-test probability of disease (PPV & 1 - NPV) for negative test result	0.221	0.066	0.008	0.004

Assuming that acutely affected pigs, highly indicative of influenza virus infection, are sampled and tested by PCR, one can be quite certain that the infection is present in the case of a positive test result. However, when the test, under the same assumption, returns negative, there is 22% probability that the disease is still present, despite the negative result.

⁷ <https://epitools.ausvet.com.au/testshome>

Positive and negative predictive value

Alternatively, one can evaluate the positive and negative predictive values of tests, for which one must first assume the prior probability of infection (some use the expected prevalence). Table 5 shows that the positive predictive value is always quite high when a highly specific test is used, even when the prior probability of infection is rather low. However, the negative predictive value is rather low when one has a high prior probability of infection (or prevalence).

Table 5 Positive and negative predictive value for test outcomes in scenarios with different prior probabilities of infection. The table is constructed using Epitools⁸, using assumed diagnostic test characteristics (Se = 0.93 & Sp = 0.99).

Scenarios for different prior probabilities of infection				
Input	High	Moderate	Low	Very Low
Prior probability of infection	0.80	0.50	0.10	0.05
Output				
Positive predictive value	1.00	0.99	0.91	0.83
Negative predictive value	0.78	0.93	0.99	1.00

From individual to multiple animals sampling

The above-mentioned examples all relate to individual sample results. One can also use an approach to calculate the probability of detecting the pathogen (P) when sampling multiple pigs. The calculation can be kept simple using $P=1-(1-d)^n$

Here, d is the prevalence (proportion of infected animals), assuming perfect test and independent, random sampling. In case that typical, clinically affected pigs are selected first, the expected prevalence in that selection of pigs is likely higher. If one cannot make a good selection based on clinical signs, one may have to consider a lower expected prevalence which may mean one has to sample more pigs (Table 6 and Figure 1). Although in that case, the presence of influenza virus may be detected in the sampled pigs, its association with clinical signs is less clear.

Although on farms the prevalence may not be very high, the prevalence value can be assumed higher in a group of pigs with characteristic clinical signs. Therefore, generally it suffices to sample 3 – 5 pigs with characteristic clinical signs to obtain a high probability of detection of infection in diseased pigs.

Note that for this formula, the assumption is that detecting one positive test result means that disease is detected. The probability of detecting at least one case becomes more likely with higher disease prevalence, larger sample sizes, and improved diagnostic sensitivity.

⁸ <https://epitools.ausvet.com.au/testshome>

Table 6. Calculated probability of detection of infection given the number of animals sampled. One or more positive test results imply having detected the infection.

Number of Animals sampled	Expected prevalence scenario			
	High $d = 0.8$	Moderate $d = 0.5$	Low $d = 0.1$	Very low $d = 0.05$
1	0.80	0.50	0.10	0.05
2	0.96	0.75	0.19	0.10
3	0.992	0.88	0.27	0.14
4	0.998	0.94	0.34	0.19
5	0.999	0.97	0.41	0.23
10	1.00	0.99	0.65	0.40

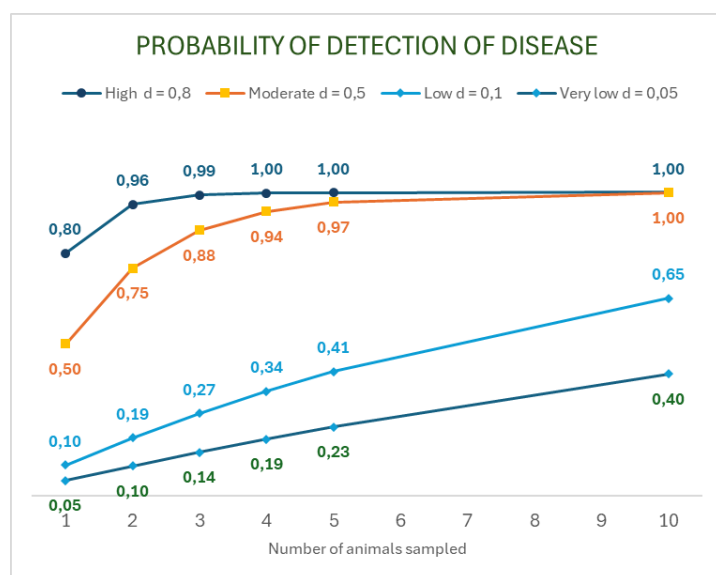


Figure 1: calculated probability of detection of a pathogen given the number of animals sampled (x-axis) and the expected pre-test prevalence (d). Important to note is that one positive test result will imply that the pathogen is present, irrespective of the number of positive samples. In other words, when you are quite confident that the pathogen is present with a high prevalence, only a few animals need to be sampled to have a high probability of detection. If there is a low expected prevalence, one needs to sample more pigs to detect the infection.