Review Article

Journal of Veterinary Diagnostic Investigation
2020, Vol. 32(4) 503–512

DOI: 10.1177/1040638720936616
jvdi.sagepub.com

Noninvasive strategies for surveillance of swine viral diseases: a review

Hanna Turlewicz-Podbielska, Jan Włodarek, Małgorzata Pomorska-Mól[1]

Abstract. In view of the intensive development of the swine industry, monitoring and surveillance of infectious diseases require low-cost, effective, and representative population sampling methods. We present herein the state of knowledge, to date, in the use of alternative strategies in the monitoring of swine health. Blood sampling, the most commonly used method in veterinary medicine to obtain samples for monitoring swine health, is labor-intensive and expensive, which has resulted in a search for alternative sampling strategies. Oral fluid (OF) is a good alternative to serum for pooled sample analysis, especially for low-prevalence pathogens. Detection of viral nucleic acids or antiviral antibodies in OF is used to detect numerous viruses in the swine population. Meat juice is used as an alternative to serum in serologic testing. Processing fluid obtained during processing of piglets (castration and tail-docking) may also be used to detect viruses. These matrices are simple, safe, cost-effective, and allow testing of many individuals at the same time. The latest methods, such as snout swabs and udder skin wipes, are also promising. These alternative samples are easy to acquire, and do not affect animal welfare negatively.

Key words: meat juice; oral fluid; processing fluid; surveillance; swine viral diseases.

Introduction

Since ~2000, there has been significant intensification of swine husbandry, which has forced changes in the surveillance of infectious pig diseases. The increase in herd size, intensive production, and globalization of the pig industry have led to the emergence of a new class of diseases characterized as comprehensive and multifactorial. Health is a key factor affecting the performance of any breeding system and it has particular importance for the swine industry. Diagnosis and surveillance of such diseases require a combination of comprehensive laboratory testing, implementation of control programs, and monitoring of their effectiveness.

Blood collection from individual animals has been the method used most commonly in veterinary practice to obtain samples for monitoring and surveying the health status of the herd. It is a very labor-intensive method, as well as stressful both for animals and the collecting personnel. Blood collection requires restraining of animals and impairs their welfare, usually requires the involvement of at least 2 people, and could be dangerous for staff taking samples, especially when collecting blood from boars, sows, or animals kept in groups. Routine surveillance based on blood sample testing is also expensive. Alternative matrices, such as oral fluid, processing fluid, meat juice, or even nasal wipes and udder skin wipes, are achieving increasing importance in the management of pig health. Unlike blood samples, such alternatives can be taken in a simple, inexpensive, safe, and noninvasive way.[12,17]

Oral fluid

Oral fluid (OF) is a mixture of secretions of salivary glands and non-salivary components: gingival secretions, expectorated bronchial and nostril secretions, blood and serum components from wounds in the mouth, bacteria and their products, viruses, fungi, exfoliated epithelial cells and other cellular components, as well as food debris.[6,63] The first mention of antibodies in OF appeared as early as 1976, when intranasal vaccination of pigs with the Thiverval strain of classical swine fever virus (CSFV) was described. Vaccination resulted in the appearance of antibodies in secretory fluids.[8] The first diagnostic use of OF was described in 2005; researchers detected the porcine reproductive and respiratory syndrome virus (PRRSV) in 20 of 24 (83%) OF samples and in 17 of 24 (71%) serum samples from finishing pigs, using reverse-transcription PCR (RT-PCR).[65]

OF contains antibodies from 2 sources: salivary glands and serum. The dominant immunoglobulin in saliva is secretory IgA, which is secreted by plasma cells in the salivary

Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Sciences, Poznań, University of Life Sciences, Poznań, Poland.

[1]Corresponding author: Małgorzata Pomorska-Mól, Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Sciences, Poznań, University of Life Sciences, Poznań, 60-637, Poland. mpomorska@up.poznan.pl

glands. It is the main immunologically active component of saliva. IgM and IgA occur in saliva at lower concentrations.[28] Antiviral antibodies as well as elements of viruses can also be detected in saliva and can be used to detect viral infections.[39] In the 1970s and 1980s, local antibody production by serum-derived plasma cells in salivary glands and DALT (duct-associated lymphoid tissue) was reported.[9,43] Evidence for the passage of serum antibodies (IgG, IgM, and IgA) to OF was found in an experiment conducted on rhesus monkeys; isotope-labeled IgG, IgM, and IgA introduced into the circulatory system of monkeys were detected in the OF after 30 min.[6] In human medicine, saliva is also an alternative to serum, mainly used in the detection of hepatitis A virus and human immunodeficiency virus.[27]

Collection of OF can be done by a trained breeder or technician, not necessarily by a veterinarian. The fact that pigs are naturally curious animals and show particular interest in easily destructible, chewy, and pliable material, enable this process.[55,76] OF can be collected from whole pens or from larger groups of pigs with the help of ropes designed for this purpose, or individual samples can be taken with the use of cotton swabs. In 2014, a method called "pathogen sampling wild animals with baits" was used to detect CSFV infection in wild boars.[39]

OF is taken most often in the form of a pooled sample from a group of animals. However, taking individual OF samples requires the involvement of only one employee, at least one fewer than in the case of blood collection. The volume of OF collected from one animal with a dry cotton swab is sufficient to perform some laboratory tests and even to preserve OF for further analysis.[17] It is also a much less stressful method for animals and is safer for the collector, especially when sampling from sows kept in groups. An important disadvantage of this method is the time required for sampling from one animal, which is twice as long as individual blood collection.[1] Usually 100% cotton, 3-strand, ~12-cm ropes are used for collecting OF from large groups of animals. It is recommended to hang ropes at the pigs' shoulder level. With animals previously experienced with this type of sampling, it is recommended to give the animals access to the rope for ~20 min, and, if OF is taken for the first time, for ~60 min.[76] Given difficulties that sometimes occur (e.g., low interest in the rope, small amounts of OF extracted from the rope), the impact has been assessed of using different flavors on the performance of OF collection; acidic or sweet supplements do not affect the efficiency of OF collection from pens.[11] Most often, the presence of only a rope is sufficient to attract pigs' attention and encourage chewing.[69,70]

The type of material from which the ropes are made has also been assessed. Synthetic ropes can more efficiently accumulate IgA found in OF, and both synthetic and cotton ropes ensure obtaining adequate amounts of IgG, the most important class of antibodies used in tests of OF.[13] Other studies have proved that raw cotton ropes are best for collecting OF.[48] The highest amount of PRRSV RT-PCR–positive samples and the highest correlation between serum and OF results was obtained by using cotton for OF collection.[30,48] IgM and IgA concentrations differed significantly in one study, depending on the collection material: cotton, hemp, or nylon rope; the lowest IgM and IgA concentrations were found in cotton, but no difference in IgG concentration was observed.[48]

The age of the animals influences the amount of fluid obtained. Sows kept in groups were less interested in ropes than growing–finishing pigs. In one study, it was not possible to collect OF from 40% of pens in which sows were located. Therefore, pen-based sampling seems to be better suited to growing pigs; it was successful in all pens containing 16- and 22-wk-old pigs.[17] It is necessary to conduct further research on the factors influencing the behavior of groups of animals in respect to chewing ropes, which could support the collection of OF and the use of this medium in sows.[17]

Although it is possible to test individual OF samples, the main application of this matrix is the analysis of pooled samples representative for many individuals. This is particularly important for monitoring the spread of low-prevalence pathogens. Individual sampling (serum) significantly increases the cost of effective surveillance, given that a relatively large number of these samples must be taken to be representative of the herd. Another important advantage of OF sampling is the minimization of stress associated with taking individual samples. However, there are some restrictions on the implementation of OF sampling. OF may contain particles from the environment (feed, feces, inorganic material) and this contamination can affect test procedures (e.g., reducing the precision of reagent distribution using a pipette).[17] Apathy, a common sign of many diseases, can lead to lack of willingness to interact with the OF collection ropes. Older animals also are less inclined to chew on the ropes. Environmental factors, such as high or low temperature, can also influence the tendency to interact with the rope. Moreover, it is not possible to collect OF with this method from very young piglets.

Oral fluid in the detection of swine viral diseases

Porcine reproductive and respiratory syndrome

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRSV (*Betaarterivirus suid 1* and *2*), is one of the diseases causing the greatest losses in modern pig production.[35] Annual PRRS losses in Europe range from an average of €75,724 on slightly affected farms during nursery and fattening periods, to an average of €650,090 if the farm was severely affected at all production stages.[44,64]

Surveillance of PRRS using OF may be based on the detection of PRRSV nucleic acid or anti-PRRSV antibodies. Most of the serum ELISAs can be modified and used to

detect antibodies in OF. This may require, for example, increasing the sample volume, extending the incubation time, changing the conjugate concentration, or changing the cutoff value.[5] PRRSV was the first virus detected in OF in pigs.[56] Since 2008, PCR and ELISAs have been specifically adapted for the detection of many porcine pathogens in OF, and since 2010, routine ELISAs have been offered for detection of anti-PRRSV antibodies in OF.[4,5]

Many studies indicate that OF is as good a matrix for detecting and monitoring PRRSV infections as is serum. Field studies, conducted in 2008, achieved 77% agreement between RT-PCR and ELISA results obtained from serum and OF.[56] The same authors also detected PRRSV in serum and OF from pigs experimentally infected with a PRRSV-2 isolate. Both serum and OF were positive in RT real-time PCR (RT-rtPCR) from day 3 after inoculation to 4–5 wk after inoculation.[56] The kinetics of changes in the amount of virus in serum and OF were similar during the experiment, but with consistently lower virus concentration in OF. Other studies were conducted on production farms in Poland; authors tested the circulation of PRRSV in a pig herd by detecting antibodies and PRRSV nucleic acid in serum and OF using ELISA and RT-PCR. PRRSV antibodies were detected in 58% of pooled serum samples and in 81% of OF samples in farm one, and 75% and 93% on the other farm, respectively.[3] Other researchers examined the usefulness of individual and pooled OF samples from growing–finishing pigs and group-housed sows and assessed the performance of commercial ELISAs using OF (OF-ELISA) and serum (SER-ELISA) by conducting tests on individual and pooled samples from 5–10 pigs. The experiment was carried out on a group of 39 growing–finishing pigs and 1,598 sows from 42 herds infected with PRRSV and from 3 herds free from PRRSV. The studies obtained a high level of OF-ELISA sensitivity compared to SER-ELISA, carried out on individual samples, pools of 5, or per pen-based samples. Individual samples, especially for sows, showed low specificity. Pooling 5 samples or using pooled samples from pens increased specificity. The dilution effect caused by false-positive samples may explain the increased specificity.[17]

However, using either OF or serum, it is difficult to differentiate maternal antibodies from those produced post-infection or post-vaccination. Given the relatively high concentration of IgG (which may be the result of passive transfer, especially in young animals) compared to other Ig isotypes, commercial ELISAs focus mainly on detecting IgG.[55] Inoculation with PRRSV-1, -2, or modified-live virus (MLV) leads to the production of IgM and IgA antibodies detectable in serum and OF.[29] Therefore later studies focused on detecting both IgA and IgM in OF. According to the authors, the use of ELISAs that simultaneously detect IgA and IgM (PRRSV IgA-IgM ELISA) is a potentially useful tool for monitoring PRRSV, especially in groups of growing piglets from PRRSV-positive or -vaccinated herds in which maternal antibodies may be present for up to 10 wk.[60]

Swine influenza

Swine influenza is caused by influenza A virus (IAV; *Orthomyxoviridae*). Influenza, especially in the endemic form, is associated with significant economic losses. In vivo direct diagnosis of influenza is based on 3 methods: virus isolation (VI), RT-PCR, or rapid point-of-care immunochromatographic tests that capture antigen. Nasal swabs (NSs) are the standard antemortem material used for these tests.[22] NS collection is a labor-intensive method; moreover, it requires careful selection of animals and collecting in the first 7 d of infection, when the virus is present in nasal secretions.[71]

The feasibility of detecting IAV using the above 3 methods in NS and OF was evaluated, and these 2 types of samples were compared in previous studies.[22] The probability of IAV VI from OF and NS was very high at 4 d post-infection (dpi); however, it decreased significantly thereafter. VI was much less successful from OF than from NS, which may be associated with a large amount of anti-IAV (IgA and IgG) antibodies in OF. However, detection of the virus by RT-PCR was possible much longer in OF than in NS; after 6 dpi, the estimated PCR cycle threshold (Ct) decreased, indicating a higher virus concentration. In these studies, Ct values in the OF were usually equal to or even lower than in NS.[22] Higher and longer detection of viral RNA in OF is likely a result of IAV replication in bronchial epithelial cells and virus excretion from the lungs by physiologic clearance mechanisms such as coughing.[32]

The sensitivity of the PCR reaction used for the detection of IAV in OF and NS has also been evaluated.[59] The average sensitivity of PCR in this evaluation was > 80% for OF. Less agreement was observed between the results obtained from OF and NS if the prevalence in the pen was < 10%. However, the probability of detecting influenza virus was 99% if the prevalence was > 18% and decreased to 69% if the prevalence was 9%. The kappa value between OF and NS results was 0.82, which indicates that OF is quite a good medium for testing the population.[59]

A commercial ELISA, originally designed to detect anti–nucleoprotein A (NP) antibodies in avian serum, has also been used to detect these antibodies in swine serum. The sensitivity and specificity of this test, carried out on swine serum, was estimated at 96.6% and 99.3%, respectively.[7] The serum ELISA procedure was modified and adapted to test 182 OF samples taken from pigs infected with the H1N1 and H3N2 subtypes of IAV. NP antibodies were detectable from 7 to 42 dpi (i.e., until the end of the experiment). Sample-to-negative (S/N) ratios for OF and serum in the same pen showed a correlation of 0.796 (Pearson correlation coefficient, $p < 0.0001$).[50] Detection of IAV NP antibodies in OF was also described in 2016. The authors compared IAV-antibody prevalence in OF pairs and pooled serum samples from pens of 3- to 20-wk-old piglets. The probability of detecting IAV-positive samples in the pen for 3- to 9-wk-old piglets was 40% and 61%, respectively, for OF and serum ($p = 0.04$);

for 10- to 14-wk-old piglets 19% and 93%, respectively, for OF and serum ($p < 0.01$); and for 18- to 20-wk-old piglets 67% and 81%, respectively, for OF and serum ($p = 0.05$).[20]

Porcine circovirus 2–associated disease

Porcine circovirus 2 (PCV-2; *Circoviridae*) is another pathogen that is responsible for significant economic losses in the global pig industry. Routine detection of PCV-2–associated disease is based on the identification of PCV-2 antigen or DNA in affected tissues in combination with the detection of characteristic histologic lesions (in situ hybridization or immunohistochemistry) in dead or killed diseased pigs. Alternative methods such as serum RT-rtPCR can be used for in vivo testing; the detection of PCV-2 and anti–PCV-2 antibodies in OF has achieved increasing importance.[46,66]

In a study of 310 pairs of sera and OF, in which the virus content in OF from PCV-2–positive pens was significantly higher than in pooled serum samples, the authors concluded that OF was well suited for detecting PCV-2 in a pen, but was not useful for determining the virus load of individual pigs.[46] Several studies confirmed the usefulness of OF in the detection of PCV-2 infections and for testing the prevalence of PCV-2 in pens.[28,56,58] Research has also shown that the amount of PCV-2 in saliva is higher, which means that detection of the virus is possible earlier and longer in OF than in serum.[19] Interpretation of rtPCR reaction results and detected amounts of PCV-2 must be carried out carefully, considering the uncertain number of animals in contact with the OF collection rope and the fact that individual animal shedding levels may vary.[24] During another study, anti–PCV-2 antibodies (IgG, IgA, and IgM isotypes) were detectable in OF from experimentally inoculated pigs from 14 dpi, and IgG and IgA isotypes were detectable at 98 dpi.[57] Although no correlation was observed between the severity of respiratory signs and the detection of PCV-2 in OF, the data indicate the possible use of pooled OF samples for additional diagnosis of porcine circoviral disease at the population level. For effective surveillance, it is recommended that the exposed population be sampled every 2–4 wk.[56]

Classical swine fever

Available data indicate that OF can also be used as a noninvasive medium in the diagnosis of classical swine fever (CSF).[40] CSFV (*Pestivirus C*) causes one of the most important transboundary swine viral diseases.

CSFV genetic material in OF has been detected in several studies performed on experimentally infected pigs. In one study, authors randomly divided a group of 8 pigs into 2 subgroups of 4 individuals. After inoculation with wild-type Alfort/Tübingen CSFV, oropharyngeal swabs (OS), blood, and OF were collected from each of the 2 groups using sampling baits containing raw cotton embedded in a cereal-based bait matrix. Virus nucleic acid was detected from 7 dpi, which coincided with the beginning of virus RNA detection in OS. The probability of detecting CSFV RNA in cotton ropes was identical or greater than in individual samples.[14] In another study, CSFV RNA was detected by multiplex RT-rtPCR (mRT-rtPCR) in OF in experimentally infected pigs from 5 dpi.[23]

Immune response in serum and OF against 2 CSFV proteins, E2 and Erns, was compared in other studies. Pigs were experimentally infected with moderately pathogenic field strain (ALD, $n = 30$) and MLV virus (LOM strain; $n = 30$). During the experiment, 1,391 OF samples and 591 serum samples, taken 14 d before infection to 28 dpi, were tested. The ELISA revealed the simultaneous appearance of E22- and Erns-specific IgG and IgA in serum and OF; IgG ELISA provided better performance. The authors concluded that the results of OF samples were consistent with those obtained using serum, and that OF can be used on a wide scale for screening a population.[49] However, cross-reactions of anti-CSFV (E2, Erns) antibodies to other pestiviruses, especially bovine viral diarrhea virus and border disease virus, are known; hence, further research is needed to develop specific antibody testing platforms that would allow proper interpretation of results.[33] The same authors compared the effectiveness of various methods using OF as a medium for testing for CSFV. Virus was detected in serum by RT-rtPCR as early as 2 dpi and as late as 28 dpi. Antigen ELISA-positive results were observed as early as 6 dpi and as late as 17 dpi. According to above-mentioned studies, RT-rtPCR was the most effective ≤10 dpi, whereas antibody detection in serum was most effective for identifying infection ≥ 14 dpi.[51] Other researchers inoculated 8 pigs intranasally with the Alfort CSFV strain. No neutralizing antibodies were found in OF during the study. CSFV nucleic acid was detectable by RT-PCR in OF from 8 dpi, which coincided with its initial detection in blood samples. The probability of detecting the virus in OF was equal or even higher compared to the corresponding blood samples.[52] The method based on OF analysis therefore requires the use of a combination of methods based on the detection of virus and antibodies to obtain the best results in monitoring herd health.

African swine fever

The causative agent of African swine fever (ASF) is the large, double-stranded DNA, enveloped African swine fever virus (ASFV; *Asfivirus*). An indirect, optimized ELISA, capable of detecting antibodies to p30 protein in the serum and OF in experimentally infected pigs was developed. Under experimental conditions, detection of antibodies was possible at 8–12 dpi. Serum ($n = 200$) and OF ($n = 200$) tests showed that OF was as good as serum as a diagnostic medium for ELISA.[21] Other authors subjected 8 pigs experimentally infected with ASFV to 2 serologic testing techniques: ELISA and immunoperoxidase test. Anti-ASFV antibodies were detectable by both techniques in OF in all animals from the early post-inoculation period to the end of the experiment.[42] The detection of ASFV genetic material by mRT-rtPCR from OF was described

by other researchers. One of them described early detection of ASFV genetic material, which is crucial to limiting the impact and spread of ASF. Genetic material of ASFV was detected as early as 3 dpi, 2–3 d before the onset of clinical disease.[23] Other studies focused on ASFV DNA survival in excretions, which is an important aspect of shedding of ASVF and infection by indirect contact with contaminated fomites. According to these studies, in OF, ASFV DNA could be detected for 35 d at 4°C, and 14 d at 12°C and 21°C. No ASFV DNA could be detected in OF after storage at 37°C.[10]

Swine vesicular disease

Swine vesicular disease virus (SVDV; *Enterovirus B*) causes highly contagious swine vesicular disease, which is clinically indistinguishable from foot-and-mouth disease and can cause huge economic losses in the swine industry.[62,78] Detection of the SVDV genome in OF using RT-PCR has been described at 1–21 dpi. The authors also isolated the virus from OF, which was possible for 1–5 dpi. Detection of anti-SVDV antibodies using a competitive ELISA based on 5B7 monoclonal antibodies was possible from 5 dpi. Moreover, the studies evaluated the kinetics of IgM and IgA concentrations in OF; IgM were detected from 6 dpi and significantly decreased at 21 dpi, and IgA appeared at 7 dpi and remained at high concentrations in OF until the end of the experiment (28 dpi). The results confirm the potential use of OF as a matrix for the detection of SVDV infection.[62]

Meat juice

Meat juice (MJ) is a mixture of serum, lymph, and released intracellular fluid. MJ can be obtained easily by thawing a meat sample several times,[53,54] and can be used as an alternative to serum in serologic testing. Sampling at the abattoir avoids the risk of disease spread associated with farm visits.[53] The disadvantage of this method is the possibility of detecting the disease only postmortem.[54] Numerous factors can affect the amount of antibodies contained in MJ: blood content in muscle tissue, level of pre-slaughter stress related to glycogen content and pH in muscles after slaughter, the level of hydration of the animal, the degree of blood supply to a given muscle, or the amount and presence of fascia in the muscle.[45,75,77] The choice of muscle for obtaining MJ is significant in terms of detection usefulness: IgG levels were higher in the myocardium and tongue, lower in limb and diaphragm muscles.[75,77] MJ can be considered as a matrix corresponding to diluted serum; therefore, there may be some discrepancies when comparing the results of the serum and MJ ELISAs. To minimize this discrepancy, a 10-fold lower dilution of MJ than serum should be used for ELISA.[45]

MJ is used as a matrix to detect pathogens in the food chain (*Salmonella* spp., *Toxoplasma gondii*).[75] In several European countries, MJ is also widely used for serologic surveillance of *Salmonella* spp. in regulated control programs.[1,36,41]

MJ can also be used as an appropriate matrix for detecting porcine viral diseases.[26] As early as 1998, studies were conducted on the use of MJ in preventive plans for monitoring pseudorabies in swine herds. A commercial ELISA for the detection of pseudorabies glycoprotein E antibodies in MJ samples was adapted for this purpose. The results obtained after analyzing 389 pairs of sera and MJ were compared; considering serum samples as a reference, individual sensitivity was 93.2% and specificity was 98.3%.[31] Satisfactory results were also obtained using MJ to detect PRRSV infection in pig herds with known PRRSV status. The herds were classified as seropositive or seronegative based on the results of a study of 10 MJ samples collected randomly in an abattoir over a period of 3 mo. The specificity of the indirect ELISA adapted to MJ was 0.98; sensitivity depended on the PRRSV strain. The authors concluded that the use of MJ can give an acceptable level of herd status classification of PRRSV infection; however, veterinarians and farmers should be aware that false-positive results may occur relatively frequently.[38]

Detection of PRRSV and the first report of the detection of PCV-2 in MJ from the shoulder muscles of wild boars from various regions in Poland was described in 2013.[16] Only one MJ sample was PRRSV positive, whereas anti–PCV-2 antibodies were found in 6 of 142 (4%) samples.[16] The sensitivity and specificity of an ELISA for PRRSV detection in MJ samples collected under experimental conditions were >95% and 100% at each dilution, respectively.[37] These studies confirmed the possibility of using MJ in PRRSV surveillance programs based on ELISAs.[37]

Use of MJ in the diagnosis of CSF in wild boars has been described. A study was carried out on 56 pigs and 21 wild boars infected or vaccinated, and 129 field samples from wild boars. The authors concluded that MJ is a suitable medium for the detection of CSFV, especially in wild boars.[56] In other studies, MJ was used to detect IgG and IgA antibodies against porcine epidemic diarrhea virus (PEDV). Correlations between S/P ratio in serum and MJ results were significant for both IG and IgA. The experiment proved the excellent performance of the PEDV IgA and PEDV IgG ELISAs in the testing of MJ, and that MJ could be used for routine surveillance of PEDV.[54] In 2019, researchers used MJ obtained from wild boar muscles to detect antibodies against Japanese encephalitis virus and hepatitis E virus; the authors tested 46 MJ samples from the diaphragm or myocardium, obtaining ELISA specificity and sensitivity of almost 100%.[77]

Processing fluid

Processing fluid (PF) consists of blood and serum obtained during castration and tail-docking (piglet processing), which is usually performed at 3–5 d old. The use of PF for the surveillance of viral diseases in breeding herds and in suckling piglets is not well studied yet, and data regarding this matrix are scarce.

PRRSV replicates in testicular epithelial cells and macrophages, which indicates that fluids obtained during processing might be an appropriate medium for detecting PRRSV.[65] Higher proportions in the presence of PRRSV-positive males with a greater amount of virus in the samples compared to females were observed. The reason for this may be the properties of this arterivirus, infection of various types of cells in the reproductive tract, and asymptomatic carriage in males. However, further research is needed on the role of sex in persistent PRRSV infections.[73] By collecting tails and testicles into separate containers, the visible amount of PF was observed only in containers of tails. Testicular fluid most likely behaves like a support medium and can wash out a small amount of blood from the tails before it clots, so collecting tails and testicles in one container is required. Dilution of the sample by the testicular fluid of PRRSV-negative males could present a risk of obtaining false-negative results when only single PRRSV-positive females are present in a litter. In this situation, there is a risk that the Ct value for RT-PCR may increase and virus load may be below the detection level.[73]

The results from PF samples were compared with those from serum in several studies. The probability of detecting PRRSV RNA in PF by RT-PCR was greater than the detection of PRRSV RNA in serum samples.[34] In other studies, the sensitivity and specificity assessment were carried out on serum and PF of seventy-seven 3-d-old piglets; the kappa value was 0.81 between results obtained from both matrixes. Sensitivity and specificity of PF results compared to serum results were 87% and 94%, respectively.[73] PF was also used for longitudinal monitoring of PRRSV status in the herds, which were subject to virus elimination. The experiment was carried out in 29 breeding herds for 65 wk; 93% of farms that had 2 consecutive negative PRRSV results in PFs using RT-rtPCR also had negative PRRSV RT-rtPCR results in the weaned piglets' serum in the same cohort.[68] The authors of the above experiments claim that PF is a valuable, effective, and economical tool for screening breeding flocks that are attempting PRRSV elimination. In another experiment, scientists compared the use of PF samples with the results obtained using OF and serum. All samples from PRRSV-negative herds were negative on RT-PCR. PRRSV was 100% detected in PRRSV-positive herd samples in all 3 matrices. The kappa value was 1, showing excellent agreement between RT-PCR results with PF, OF, and serum.[2] The results of other studies reporting the detection of PRRSV RNA in PF indicated that the use of PF in monitoring disease provides reliable results.[25,61,67]

Not much is known about the impact of pooling PF samples on the ability of PCR to correctly classify a sample as positive. Authors have estimated that aggregation of at least 50 litters was possible when the PCR Ct in the sample was ~ 22, and up to 40 litters if the Ct value was ~ 33. Pooling did not affect the results of the PCR reaction if the initial Ct values were ~ 20 to ~ 25. For litters with baseline Ct ≥ 30, the number of samples in the pool should be limited. The results of these tests provide a general framework that can help in more detailed analysis and interpretation of the results of monitoring tests carried out using PF—the study was conducted only with 2 strains of PRRSV-2 from 2 farms recently infected with PRRSV.[74]

PF is a promising, practical, and inexpensive matrix, the use of which can improve the monitoring of PRRSV. It allows the testing of more piglets more frequently, which increases the likelihood of detecting the virus with a minimal prevalence. This matrix is particularly easy to obtain in 3- to 5-d-old piglets, when it is not possible to collect OF. Collecting blood samples from such young piglets is also fraught with higher risk than in older animals. Moreover, testing PF allows the rapid classification of piglets as PRRSV-negative or -positive. However, this method does not reveal how many piglets from the pooled sample are positive. There is also the possibility of environmental contamination of samples during processing. The possibility of using PF in the monitoring of other swine viral diseases requires more comprehensive research.

Nasal wipes and udder skin wipes

Implementation of new, cost-effective, and non-disturbing animal welfare methods to collect representative samples of the population is also resulting in the investigation of new matrices: nasal wipes (NWs, also known as snout wipes).

NWs are collected using cotton gauze, which is wiped across the snout, collecting secretions from the snout and nares.[47] The usefulness of cotton for this purpose is debatable: it is assumed that cotton contains PCR inhibitors,[72] but cotton ropes are the gold standard for OF collection. There are reports that although fewer viral particles are detected in cotton by RT-rtPCR compared to polyester swabs, cotton gauzes work very well for IAV detection in field studies.[20] As mentioned earlier, NSs are the gold standard in IAV detection; however, collection of NSs requires animal restraining, training, and at least 2 employees involved. In contrast, collecting snout wipes requires no special training, is simple, and minimizes the stress on animals, and requires fewer employees involved than NS collection.[52] It is also highly acceptable in public opinion.[15,47] NW sampling allows for a quick assessment of the individual pig's health.

The possibility of IAV RNA detection and VI of IAV in material obtained from NWs has been described, which gives this matrix a certain advantage over OF, from which VI is rarely possible. However, there are some restrictions that should be kept in mind when using NWs. Given the contact of the swine snout with the external environment, NS sampling is often associated with the simultaneous collection of many contaminants (litter, food, feces, etc.) that can inhibit the PCR reaction or be toxic to cells when attempting to isolate the virus. Furthermore, the detection of IAV may be the result of environmental pollution and may not always be the result of active shedding by the animal. NW storage in a

freezer or refrigerator for transport also requires 3–4 times more space than NSs. Storing samples for a long time at room temperature, refrigerated, or frozen and thawed, as well as overheating the sample during thawing, results in reduced virus viability.[15]

Several studies suggest concordance for IAV detection using RT-rtPCR and VI from NW and NS. The first experiment was conducted on 553 paired NWs and NSs, in which 517 of 553 (93.5%) RT-rtPCR results and 511 of 533 (92.4%) VI results agreed. The estimated sensitivity of RT-rtPCR and VI for NWs compared to NSs was 92.9% and 82.9%, respectively.[15] In the second study, authors also used NWs; 49 of 90 (54%) and 51 of 90 (57%) positive NS and NW samples were detected, respectively.[18] The same authors later compared the usefulness of different matrices, including NWs among others; in fattening farms, the kappa coefficient (0.64) showed significant agreement in the detection of IAV by RT-rtPCR in pooled NS and NW samples.[72]

Udder skin wipes (USWs) allow assessment of the health status of the litter before weaning. Secretions from the oral and nasal cavities in suckling piglets are deposited on the skin of the sow`s udder.[72] Information about the role of the USW in monitoring viral diseases occurring in litters of suckling piglets is very scarce. Researchers compared 8 different matrices (NSs; NWs, and oropharyngeal swabs; OF; surface wipes, and USWs; airborne particles deposited on surfaces, and air samples) among >1,300 samples from breeding farms and wean-to-finish facilities, and found that USWs together with oropharyngeal swabs were the best matrices for isolation of IAV from suckling piglets. USWs also proved to be suitable for virus detection by RT-rtPCR.[18] Detection and monitoring of PRRSV in the breeding herd using USWs was reported for the first time in 2019. The authors obtained a sensitivity of 43% and a specificity of 98% in their studies on USWs, comparing the results obtained from serum (gold standard). The kappa coefficient during processing time was 0.49. The percentage agreement between the results obtained from USWs and serum was 81.8%. PRRSV was detected at processing in USWs, in environmental wipes and airborne-deposited particle samples up to 14 wk post-outbreak, and at weaning in USWs up to 17 wk post-outbreak.

Ease of collection, minimizing stress, and improved animal welfare support the use of NWs and USWs in commercial herds for surveillance of viral diseases. NWs are a good alternative to NSs. Both the use of NWs to detect other respiratory pathogens in pigs and the use of SWs in the monitoring and detection of infectious diseases require further study.

Conclusion

Serum, as well as NSs, tissue, and organ samples are used most often in the diagnosis of swine viral diseases. However, these methods are costly and labor-intensive. Moreover, they can be hazardous for both animals and the collector and often require the restraint of animals. Some of these samples can only be obtained postmortem. These problems raise the need for new strategies for sampling and monitoring animal health. Alternative matrices have many advantages over traditional sampling methods. These matrices, in particular OF and PF, offer an opportunity to simplify costly sampling procedures. In the era of ever-increasing requirements for monitoring the health of larger herds, alternative matrices allow for quick assessment of the health status of the herd, and assessment of the effectiveness of implemented eradication methods. Moreover, these methods do not affect animal welfare negatively. The use of alternative matrices requires validation and confirmation of the results obtained in comparison with reference tests.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The scientific activity of M. Pomorska-Mól was supported by statutory funding 506.514.05.00 of the Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Science, Poznań, University of Life Sciences, Poznań, Poland. The scientific activity of H. Turlewicz-Podbielska was supported by grant 506.514.05.00 of the Young Researcher Program of the Faculty of Veterinary Medicine and Animal Science Poznań, University of Life Sciences, financed by the Polish Ministry of Science and Higher Education.

ORCID iDs

Hanna Turlewicz-Podbielska https://orcid.org/0000-0002-4509-8852
Małgorzata Pomorska-Mól https://orcid.org/0000-0003-2220-2730

References

1. Alban L, et al. The new classification system for slaughter-pig herds in the Danish *Salmonella* surveillance-and-control program. Prev Vet Med 2002;53:133–146.
2. Baliellas J, et al. Active surveillance of PRRSV in breeding, nursery and finishing farms from carcasses. Abstracts of European Symposium on Porcine Health Management; 2019; The Netherlands. [Cited 2020 June 2]. https://eaphm.org/porcine-health-management?f%5B0%5D=download_topic_filter%3A192&search_api_fulltext=&page=4
3. Biernacka K, et al. Detection of porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) in oral fluid of pigs. Res Vet Sci 2016;109:74–80.
4. Bjustrom-Kraft J, et al. The use of oral fluid diagnostics in swine medicine. J Swine Health Prod 2018;26:262–269.
5. Carmeron SO, Carman WF. The use of OraSure ® collection device for hepatitis virus testing in health care settings. J Clin Virol 2005;34:22–28.
6. Challacombe S, et al. Passage of immunoglobulins from plasma to the oral cavity in rhesus monkeys. Immunology 1978;35:923–931.

7. Ciacci-Zanella JR, et al. Detection of anti-influenza A nucleoprotein antibodies in pigs using a commercial influenza epitope-blocking enzyme-linked immunosorbent assay developed for avian species. J Vet Diagn Invest 2010;22:3–9.
8. Corthier G. Swine fever: influence of passive immunity on pig immune response following vaccination with a live virus vaccine (Thiverval strain). Ann Vet Res 1976;7:361–372.
9. Crawford J, et al. Minor salivary glands as a major source of secretory immunoglobulin A in the human oral cavity. Science 1975;190:1206–1209.
10. Davies K, et al. Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 isolate. Transbound Emerg Dis 2015;64:425–431.
11. Dawson LL, et al. The effects of flavored rope additives on commercial pen-based oral fluid yield in pigs. J Vet Behav 2015;10:267–271.
12. De Lucia A, Edwards SA. Matrici alternative al sangue per la sorveglianza delle malattie trasmissibili del suino [Oral fluids, meat juice, and processing fluids: non-invasive alternative diagnostic medium for disease monitoring in pigs]. Large Anim Rev 2019;25:25–33. Italian.
13. Decorte I, et al. Detection of total and PRRSV-specific antibodies in oral fluids collected with different rope types from PRRSV-vaccinated and experimentally infected pigs. BMC Vet Res 2014;10:134.
14. Dietze K, et al. Rope-based oral fluid sampling for early detection of classical swine fever in domestic pigs at group level. BMC Vet Res 2017;13:5.
15. Edwards JL, et al. Utility of snout wipe samples for influenza A virus surveillance in exhibition swine populations. Influenza Other Respir Viruses 2014;8:574–579.
16. Fabisiak M, et al. Detection of porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in meat juice samples from Polish wild boar (*Sus scrofa* L.). Acta Vet Hung 2013;61:529–536.
17. Fablet C, et al. Oral fluid versus blood sampling in group-housed sows and finishing pigs: feasibility and performance of antibody detection for porcine reproductive and respiratory syndrome virus (PRRSV). Vet Microbiol 2017;204:25–34.
18. Garrido-Mantilla J, et al. Comparison of individual, group and environmental sampling strategies to conduct influenza surveillance in pigs. BMC Vet Res 2019;15:61.
19. Gass-Cofré A, et al. Introduction of PCV2-vaccination in a sow farm and effects on virus load in blood and saliva samples. Abstracts of European Symposium on Porcine Health Management; 2019; The Netherlands. [Cited 2020 June 2]. https://eaphm.org/document/download/85f52314-6e15-42e9-b8e5-97917049c67f/3359
20. Gerber PF, et al. Using oral fluids samples for indirect influenza A virus surveillance in farmed UK pigs. Vet Med Sci 2016;3:3–12.
21. Giménez-Lirola LG, et al. Detection of African swine fever virus antibodies in serum and oral fluid specimens using a recombinant protein 30 (p30) dual matrix indirect ELISA. PLoS One 2016;11:e0161230.
22. Goodell CK, et al. Probability of detecting influenza A virus subtypes H1N1 and H3N2 in individual pig nasal swabs and pen-based oral fluid specimens over time. Vet Microbiol 2013;166:450–460.
23. Grau FR, et al. Detection of African swine fever, classical swine fever, and foot-and-mouth disease viruses in swine oral fluids by multiplex reverse transcription real-time polymerase chain reaction. J Vet Diagn Invest 2015;27:140–149.
24. Hernandez-Garcia J, et al. The use of oral fluids to monitor key pathogens in porcine respiratory disease complex. Porcine Health Manag 2017;3:7.
25. Johnson C, et al. Breed-to-wean farm performance and PRRSV RNA detection in processing fluids over time on a PRRS naive herd vaccinated with MLV vaccine. Abstracts of European Symposium on Porcine Health Management; 2019; The Netherlands. Accessed 2020.06.02: https://eaphm.org/document/download/26b597d5-2115-460d-a0d2-3fc86806bb84/3168
26. Kaden V, et al. Meat juice as diagnostic sample for virological and serological diagnosis of classical swine fever. Dtsch Tierarztl Wschr 2009;116:173–179.
27. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. Crit Rev Oral Biol Med 2002;13:197–212.
28. Kim WI. [Application of oral fluid sample to monitor porcine circovirus-2 infection in pig farms]. J Vet Clin 2010;27:704–12. Korean.
29. Kittawornrat A, et al. Kinetics of the porcine reproductive and respiratory syndrome virus (PRRSV) humoral immune response in swine serum and oral fluids collected from individual boars. BMC Vet Res 2013;9:61.
30. Kittawornrat A, et al. Porcine reproductive and respiratory syndrome virus (PRRSV) in serum and oral fluid samples from individual boars: will oral fluid replace serum for PRRSV surveillance? Virus Res 2010;154:170–176.
31. Le Potier MF, et al. Use of muscle exudates for the detection of anti-gE antibodies to Aujeszky's disease virus. Vet Rec 1998;143:385–387.
32. Levandowski RA, et al. Modifications of lung clearance mechanisms by acute influenza A infection. J Lab Clin Med 1985;106:428–432.
33. Loeffen WL, et al. Seroprevalence and risk factors for the presence of ruminant pestiviruses in the Dutch swine population. Vet Microbiol 2009;136:240–245.
34. Lopez W, et al. Porcine reproductive and respiratory syndrome monitoring in breeding herds using processing fluids. J Swine Health Prod 2018;26:146–150.
35. Lunney JK, et al. Porcine reproductive and respiratory syndrome virus (PRRSV): pathogenesis and interaction with the immune system. Ann Rev Anim Biosci 2016;4:129–154.
36. Merle R, et al. Serological *Salmonella* monitoring in German pig herds: results of the years 2003–2008. Prev Vet Med 2011;99:229–233.
37. Molina RM, et al. Diagnostic performance of assays for the detection of anti-porcine reproductive and respiratory syndrome virus antibodies in serum and muscle transudate ('meat juice') based on samples collected under experimental conditions. J Vet Diagn Invest 2008;20:735–743.
38. Mortensen S, et al. Monitoring porcine reproductive and respiratory syndrome virus infection status in swine herds based on analysis of antibodies in meat juice samples. Vet Res 2001;32:441–453.
39. Mortimer PP, Parry JV. The use of saliva for viral diagnosis and screening. Epidemiol Infect 1988;101:197–201.

40. Mouchantat S, et al. Novel rope-based sampling of classical swine fever shedding in a group of wild boar showing low contagiosity upon experimental infection with a classical swine fever field strain of genotype 2.3. Vet Microbiol 2014;170:425–29.
41. Mousing J, et al. Nation-wide *Salmonella enterica* surveillance and control in Danish slaughter swine herds. Prev Vet Med 1997;29:247–261.
42. Mur L, et al. Potential use of oral fluid samples for serological diagnosis of African swine fever. Vet Microbiol 2013:165: 135–139.
43. Nair P, Schroeder H. Duct associated lymphoid tissues (DALT) of minor salivary glands and mucosal immunity. Immunology 1986;57:171–180.
44. Nathues H, et al. Cost of porcine reproductive and respiratory syndrome virus at individual farm level—an economic disease model. Prev Vet Med 2017;142:16–29.
45. Nielsen B, et al. Use of muscle fluid as a source of antibodies for serologic detection of *Salmonella* infection in slaughter pig herds. J Vet Diagn Invest 1998;10:158–163.
46. Nielsen GB, et al. Comparison of serum pools and oral fluid samples for detection of porcine circovirus type 2 by quantitative real-time PCR in finisher pigs. Porcine Health Manag 2018;4:2.
47. Nolting JM, et al. Nasal wipes for influenza A virus detection and isolation from swine. J Vis Exp 2015;106:53313.
48. Olsen C, et al. Effect of collection material and sample processing on pig oral fluid testing results. Vet J 2013;198: 158–163.
49. Panyasing Y, et al. Detection of classical swine fever virus (CSFV) E2 and E^{rns} antibody (IgG, IgA) in oral fluid specimens from inoculated (ALD strain) or vaccinated (LOM strain) pigs. Vet Microbiol 2018;224:70–77.
50. Panyasing Y, et al. Detection of influenza A virus nucleoprotein antibodies in oral fluid specimens from pigs infected under experimental conditions using a blocking ELISA. Transbound Emerg Dis 2012;61:177–184.
51. Panyasing Y, et al. Effective surveillance for early classical swine fever virus detection will utilize both virus and antibody detection capabilities. Vet Microbiol 2018;216: 72–78.
52. Petrini S, et al. Detection of classical swine fever virus infection by individual oral fluid of pigs following experimental inoculation. J Vet Diagn Invest 2017;29:254–257.
53. Pińeiro M, et al. Meat juice: an alternative matrix for assessing animal health by measuring acute phase proteins. Correlations of pig-MAP and haptoglobin concentrations in pig meat juice and plasma. Res Vet Sci 2009;87:273–276.
54. Poonsuk K, et al. Detection of porcine epidemic diarrhea virus (PEDV) IgG and IgA in muscle tissue exudate ("meat juice") specimens. Porcine Health Manag 2018;4:31.
55. Prickett J, et al. Detection of porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. J Vet Diagn Invest 2008;20:156–163.
56. Prickett JR, et al. Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections. J Swine Health Prod 2008;16:86–91.
57. Prickett JR, et al. Prolonged detection of PCV2 and anti-PCV2 antibody in oral fluids following experimental inoculation. Transbound Emerg Dis 2011;58:121–127.
58. Ramirez A, et al. Efficient surveillance of pig populations using oral fluids. Prev Vet Med 2012;104:292–300.
59. Romagosa A, et al. Sensitivity of oral fluids for detecting influenza A virus in populations of vaccinated and non-vaccinated pigs. Influenza Other Resp 2012;6:110–118.
60. Rotolo ML, et al. Detection of porcine reproductive and respiratory syndrome virus (PRRSV)-specific IgM-IgA in oral fluid samples reveals PRRSV infection in the presence of maternal antibody. Vet Microbiol 2017;214:13–20.
61. Sanhueza J, et al. Evaluation of parity as a delaying factor to reach PRRSV stability in sow farms. Abstracts of European Symposium on Porcine Health Management; 2019; The Netherlands. Accessed 2020.06.02: https://eaphm.org/document/download/c138e780-6bf0-4eee-ab2d-cdc-22ca0ebb7/3108
62. Senthilkumaran C, et al. Use of oral fluids for detection of virus and antibodies in pigs infected with swine vesicular disease. Transbound Emerg Dis 2017;64:1762–1770.
63. Simer R, et al. An improved method for PRRS virus surveillance and monitoring. Proc International PRRS Symposium; 2005; St. Louis, MO. Accessed 2020.06.02: https://www.pork.org/wp-content/uploads/2008/07/05-146-ZIMMERMAN-ISU.pdf
64. Stoian A, Rowland R. Challenges for porcine reproductive and respiratory syndrome (PRRS) vaccine design: reviewing virus glycoprotein interactions with CD163 and targets of virus neutralization. Vet Sci 2019;6:9.
65. Sur JH, et al. Porcine reproductive and respiratory syndrome virus replicates in testicular germ cells, alters spermatogenesis, and induces germ cell death by apoptosis. J Virol 1997;71:9170–9179.
66. Szczotka-Bochniarz A, Podgórska K. Porcine circovirus type 2 infections in pigs: current terminology, clinical syndromes, and diagnostic criteria. Med Weter 2015;71:88–93.
67. Trevisan G, et al. Applications of population-based methods for PRRS monitoring and surveillance in breeding herds undergoing virus elimination. Abstracts of European Symposium on Porcine Health Management; 2019, Netherlands. Accessed 2020.06.02: https://eaphm.org/document/download/1914cf78-e002-4980-abad-90e5d91232c8/3075
68. Trevisan G, et al. Use of processing fluid samples for longitudinal monitoring of PRRS virus in herds undergoing virus elimination. Porcine Health Manag 2019;5:18.
69. Trickett SL, et al. The role of novelty in environmental enrichment for the weaned pig. Appl Anim Behav Sci 2009;116: 45–51.
70. Van de Weerd HA, Day JE. A review of environmental enrichment for pigs housed in intensive housing systems. Appl Anim Behav Sci 2009;116:1–20.
71. Van Reeth K, et al. Influenza virus. In: Zimmerman JJ, et al., eds. Diseases of Swine. 10th ed. Wiley, 2012:557–572.
72. Vilalta C, et al. Indirect assessment of porcine reproductive and respiratory syndrome virus status in pigs prior to weaning by sampling sows and the environment. Vet Microbiol 2019;237:108406.

73. Vilalta C, et al. Use of processing fluids and serum samples to characterize porcine reproductive and respiratory syndrome virus dynamics in 3 day-old pigs. Vet Microbiol 2018;225:149–156.
74. Vilalta C, et al. Effect of litter aggregation and pooling on detection of porcine reproductive and respiratory virus in piglet processing fluids. J Vet Diagn Invest 2019;31:625–628.
75. Wallander C, et al. "Meat juice" is not a homogeneous serological matrix. Foodborne Pathog Dis 2015;12:280–288.
76. White D, et al. Recommendations for pen-based oral-fluid collection in growing pigs. J Swine Health Prod 2014:22:138–141.
77. Yonemitsu K, et al. Detection of anti-viral antibodies from meat juice of wild boars. J Vet Med Sci 2019;81:155–159.
78. Zhang G, et al. Molecular evolution of swine vesicular disease virus. J Gen Virol 1999;80:639–651.