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Mini Review

OVERVIEW OF NON-INVASIVE SAMPLING METHODS USED IN INTENSIVE SWINE FARMING

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Abstract

Monitoring the health of swine herds is essential to ensure good manufacturing practices. Traditionally, active and passive surveillance on farms involved invasive sampling methods, where specific animals were selected, restrained, and sampled. However, with the increasing intensity of swine production, alternative methods for effective herd surveillance became necessary.

Non-invasive sampling provides a convenient and cost-effective approach to monitor the entire herd without compromising animal welfare, while still obtaining suitable samples for testing. Oral fluids have been widely used in both human and livestock health surveillance for various viral pathogens, including significant diseases. Nasal wipes (NW) utilize different cloth materials soaked in phosphate-buffered saline (PBS) or tissue culture medium with antibiotics and antimycotics to sample for swine influenza virus (SIV). Udder skin wipes (USW) offer an alternative method to assess the health status of piglets in a litter. During routine procedures such as tail docking and castration, a mixture of blood and serum can be collected, known as process fluids (PF), which has proven successful in monitoring herds for the presence of porcine reproductive and respiratory syndrome virus (PRRSV). Furthermore, air sampling

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has emerged as a novel technique to detect pathogens in various farming systems and animal species. This method offers the advantage of obtaining diagnostic samples without direct animal contact.

By employing these non-invasive sampling methods, swine producers can implement effective surveillance strategies while maintaining animal welfare standards and obtaining reliable diagnostic information.

Key Words: air samples, non-invasive sampling, nasal wipes, oral fluid, processing fluid, udder skin wipes

Disease monitoring of swine herds is necessary for the maintenance of good manufacturing practices. Active and passive surveillance on farms is primarily conducted by using traditional sampling methods, which implies singling out animals that would be subsequently restrained and usually sampled by invasive methods. This type of monitoring relies upon sampling enough animals to cover an entire herd based on the mathematical probability of disease presence. During sampling, each animal has to be restrained, which is often stressful, and as such can lead to a decrease in production (Grandin and Shivley, 2015; Turlewicz-Podbielska et al., 2020). By handling animals, the workers are exposed to possible injury, which is especially the case when dealing with large boars or sows with piglets, or when entering pens with a large number of animals inside (Turlewicz-Podbielska et al., 2020). In animals that are sampled and handled frequently, cumulative stress can lead to production losses and economic losses for the farms (Martínez-Miró et al., 2016). Traditional sampling procedures incur a high monetary toll on farm owners since each animal is sampled individually, and multiple samples are needed in order to understand the disease status of a herd (Turlewicz-Podbielska et al., 2020). Additionally, in industrial swine and poultry production, the herd is monitored, not the individual animal.

Non-invasive sampling strategies were primarily developed for sampling difficult-to-reach wild animals (Schilling et al., 2022). With the intensifying swine production, additional methods for adequate herd surveillance had to be developed (Turlewicz-Podbielska et al., 2020). Swine production has increased by almost 80% worldwide since 1968 (Food and Agriculture Organisation of the United Nations, 2020), while the production shifted from small-scale backyard farms to primarily large industrial complexes (Henao-Diaz et al., 2017). This led to an increase of animals on a relatively small surface area, allowing for quick and easy disease spread. In such farm holdings, the introduction of new animals and human movement through the farm can result in novel disease spread or emergence (Henao-Diaz et al., 2017; Kinsley et al., 2019). Non-invasive sampling offers an easy and cost-effective way of monitoring the entire herd, without contributing to a decrease in animal welfare, but while still providing adequate samples for testing (Turlewicz-Podbielska et al., 2020). Different sampling techniques have been developed, utilizing different matrices, such as oral fluids, meat

juice, udder swabs, nasal swabs, and even air sampling, while still achieving results comparable to traditional sampling techniques (blood, serum etc.)

Swine viral diseases cause significant economic loss in swine production. It has been estimated that porcine respiratory and reproductive syndrome (PRSSV) causes US$668 million in damages annually in swine production in the USA, while in Denmark, damages have been evaluated at €126 per sow (Dee et al., 1997; Nieuwenhuis et al., 2012). A similar situation can be observed when considering the swine influenza virus (SIV), where economic losses have been estimated between US$ 3-10 per pig produced (Calderón Díaz et al., 2020). Adequately and swiftly collected samples, which minimize the potential for disease spread by farm workers, are necessary to lower swine production costs.

The goals of this review paper are to present alternative non-invasive sampling methods that can be used in intensive swine production for monitoring viral swine pathogens, and to raise awareness among veterinary farm professionals of the implementation and possible use of these methods.

1. Oral fluid

Oral fluid (OF), as the name implies, is gathered from the oral cavity and is a mixture of saliva, fluid from the upper part of the respiratory tract, nasal secretions, food components, oral microbiome, wound detritus, and different cellular components. Through the salivary glands, immunoglobulins IgA and IgM are secreted. Their use in diagnostics was first reported by Corthier et al. (1976) while conducting vaccination trials against classical swine fever, when they used OF to measure the immune response. OFs have been used for decades now, in both human health surveillance and in livestock, for different viral pathogens including some of the most important diseases (Prickett and Zimmerman, 2010).

OF is gathered from swine using cotton ropes, which are tied at the ends of pens, and given to the swine to chew. Different types of ropes can be used, and Henao-Diaz et al. (2017) suggests using ropes 1.6 cm in diameter for larger swine categories, and 0.8 cm for piglets. The optimum exposure duration for collection is 30 min, although if swine are sampled for the first time, the collection time should be increased upwards of 45 min up to one hour (Almeida et al., 2018; Boulbria et al., 2020; Ramirez et al., 2012). Due to their natural inquisitive nature, swine explore their surroundings by chewing unknown objects, which is especially the case in younger age categories. OF can be collected from all swine production categories although there are differences in technique. In order to gather OF from boars or sows, Brent et al. (2015) suggested training them, by repeated exposure to cotton ropes for 20 minutes a day, to ensure adequate sampling (Henao-Diaz et al., 2017).

After the samples have been collected, they should be placed in water-proof biosafety bags, chilled at 4 °C, and transported to the laboratory as soon as possible. To improve detection, and allow for simpler pipetting, the samples should be centrifuged. Different

centrifugation protocols have been reported. Kittawornrat et al. (2013) published a protocol for porcine reproductive and respiratory syndrome virus (PRRSV) detection in OF collected after 12000 x g for 8 hours' centrifugation, while the same author also published a study in which samples were centrifuged at 14000 x g for 30 sec Kittawornrat et al. (2014). In general, centrifugation at 3000-15000 x g for 5-15 minutes has been successful in removing large particles, and allowing for better pipetting and antibody and nucleic acid detection (Henao-Diaz et al., 2017). OF collection is simple and can be done by trained farm personnel, while veterinary assistance is not required, saving time and money for the farm owners.

One of the biggest breakthroughs for OF was when the first commercial tests for antibodies against the human immunodeficiency virus (HIV) were developed in 1995 by Prickett and Zimmerman (2010). IgM antibodies are the first to appear in the serum, but they have a short half-life and are not usually utilized for routine diagnostics (Mestecky, 1993). IgA is secreted from plasma cells and is the biggest antibody category in OF (Mestecky, 1993). IgG appears in OF after IgA, and its half-life appears to be the longest, which is why they are primarily targeted in different immunological tests (Chiappin et al., 2007; Kittawornrat et al., 2013, 2012). Although IgA, IgM, and IgG can be detected in OF, it should be kept in mind that their concentrations are significantly lower than in the serum (Olsen et al., 2013). Antibody detection in OF has been successfully used to detect antibodies against some of the most important swine diseases, including African and classical swine fever, PRRSV, SIV, and porcine circovirus type 2 (PCV-2) (Giménez-Lirola et al., 2016; Kittawornrat et al., 2013, 2012; Panyasing et al., 2018, 2013; Ramirez et al., 2012).

Besides antibody detection, viral nucleic acid of viruses that are excreted through saliva can also be detected. The primary method of detection is usually PCR. Detection rates in OF are dependent upon the viral kinetics, and excretion periods, and as such, viruses whose primary excretion route is saliva will be more easily detected (pseudorabies virus, SIV, foot and mouth disease virus (FMDV), etc.). Since OF are collected in a contaminated environment, and come into contact with the sides of the pen, other pathogens that are not excreted orally can also be detected, such as PCV-2 or porcine epidemic diarrhea virus, which are excreted through fecal routes (Bjustrom-Kraft et al., 2016; Woźniak et al., 2019).

The negative aspects of using OF for viral detection include primarily a lower viral amount, which can hamper diagnostics especially when considering sample pooling. Since OF are not collected through a sterile process and the fluid comes into contact with the environment, other potential pathogens can be recovered that are not naturally excreted through saliva. However, the above-mentioned environmental contamination can be a deterrent, causing possible tissue culture contamination or toxicity, and as such this limits the detection capacity of OF samples.

2. Nasal wipes and udder skin wipes

As previously mentioned, SIV causes significant economic damage in the swine industry. SIV belongs to the group of influenza A viruses, all of which are very similar and characterized by a high level of mutation and reassortment, allowing for the potential development of highly pathogenic strains (International Committee for the Taxonomy of Viruses, 2009). Swine are regarded as a mixing vessel for different strains of the influenza A virus, and as such represent a threat to humans (Ma et al., 2008). For these reasons, simple and reliable sampling strategies have been developed.

One of the most reliable methods described has been the collection of nasal swabs (NS), which allow for the collection of nasal discharge directly from the nasal canal. Although this method is reliable, swine have to be restrained by trained personnel, and only then can swabs be collected. Even though this method is less invasive than the traditional sampling method, the restraining process often causes loud noise, and as such is disturbing to other swine, creating a stressful environment (Nelson et al., 2018). Novel methods for SIV sampling have been considered, such as nasal wipes (NW), which use different cloth materials, drenched in phosphate-buffed saline (PBS) or tissue culture medium with antibiotics and antimycotics for sampling (Nelson et al., 2018; Nolting et al., 2015). This method does not require the restraining of swine and can be performed by farm personnel. Different materials have been used for NW, such as cotton, polyester, or mixed polyester fabrics (Nelson et al., 2018). For molecular testing, the best results have been achieved with wipes made of cotton as reported by Nelson et al. (2018), although Vilalta et al. (2019b) report possible PCR inhibitors in cotton, even though cotton swabs and cotton ropes for oral fluids have been used for decades, often as the golden standard for sample collecting (Turlewicz-Podbielska et al., 2020). Nelson et al. (2018) reported lower viral viability in cotton compared to polyester or mixed polyester wipes, and as such, different types of wipes can be used depending on the sampling goal. However as Edwards et al. (2014) reported, cotton gauze is the easiest to use, since it already comes in sterile packages and is widely available.

After the NW are collected, they should be transported to the laboratory either frozen or at 4 °C. Edwards et al. (2014) suggests centrifuging the samples for 30 min at 1200 x g before using the supernatant for molecular methods. Negative aspects of NW should also be considered, primarily the lower viability of the targeted virus either with respect to environmental contamination or the material cytotoxicity, or just the low viral load on the surface of the nasal plane. The above-mentioned factors can also influence molecular diagnostics, as lower viral loads can lead to lower Ct values, and also limit pooling of samples.

Udder skin wipes (USW) are an alternative sampling method that can be used to assess the health status of a litter. USW, similar to NW, can be made from different materials, drenched in PBS or tissue culture medium with antibiotics and antimycotics. Samples are collected from sows with suckling piglets and represent a sample of piglet saliva,

nasal secretions, and the environmental particles that can be found on the udder. USW have been successfully used for monitoring the health status of swine for PRRS and SIV (de Lara et al., 2022; Vilalta et al., 2021, 2019b). One USW represents a sample from the entire litter, and de Lara et al. (2022) does not recommend pooling more than three samples of USW. According to Garrido-Mantilla et al. (2019), USW should be collected during or after suckling, so to collect as much as possible of the piglet's secretions. Samples should be either frozen or chilled and as such transported to the laboratory. Further processing should include centrifugation as previously described for NW. The supernatant should be decanted and stored at -80 °C until further use.

3. Processing fluid (PF)

During piglet processing, tail docking, and castration of piglets, a mixture of blood and serum is produced. These fluids, collectively termed process fluids (PF), have been used successfully for monitoring herds for the presence of PRRSV (Vilalta et al., 2021). Vilalta et al. (2021) compared OF, tail docking fluid and testicular fluid collected from piglets for the detection of PRRSV, and found that testicular fluid had the best overall score with an average positive predictive value of 85% and an average negative predictive value of 90% (Vilalta et al., 2021). The lowest scores were obtained by using the USW, with the average positive predictive value at 100%, but the average negative predictive value significantly lower at 52% (Vilalta et al., 2021). Trevisan et al. (2019) conducted a study on a farm infected with PRSSV and found PF practical and useful for assessing the health status of a herd.

However, the negative aspects of PF should also be considered. Vilalta et al. (2019a) estimated that 50 litters could be aggregated if there were a PRRSV-positive piglet with a Ct value under 22. However, if the Ct value was approximately 33, then 40 piglet litters could be tested. Vilalta et al. (2019a) also considered pooling of samples, and suggested that pooling of samples with Ct values between 20-25 would not change the Ct values, but that pooling of samples with Ct values above 30 should be reduced. However, these results should be considered as a guideline for result interpretation, and not a sampling guide, since the number of viral particles in the potentially -positive sample used for surveillance cannot be known. de Almeida et al. (2021) suggested using different sampling methods on a regular basis (at processing and weaning) in order to minimize possible false positives and achieve control or freedom of disease.

PF is a novel sampling method for viral detection in swine herds, and this matrix is easy to obtain from piglets aged three to five days. Since castration and tail docking is a standard procedure on swine farms, it does not require any additional personnel training, which makes PF simple to apply on a farm holding. Sampling PF also does not require any additional handling of piglets or blood drawing, which is difficult in young piglets.

4. Air samples

Since diseases transported by air are notoriously difficult to control, sampling methods that collect air as a herd sample have been developed. One of the first reports of the successful use of air samplers was for the detection of FMDV in swine by using a large-volume cyclone air sampler (Donaldson et al., 1982). The experiment was conducted on diseased swine in the acute stage of the disease in an enclosed space and showed promising results (Donaldson et al., 1982). Since then, different air samplers have been developed that can be classified based on the size of the particles and the air volume that the apparatus uses. Colenutt et al. (2016) tested the BioBadge 100 (ICx Technologies, VA, USA) with an airflow capacity of 35 L per min and size capacity for 1-10 μm particles, the BioCapture 650 (MesoSystems, NM, USA) with airflow of 200 L per min, the SKC BioSampler (SKC Inc. PA, USA), which is a glass liquid sampler with airflow at 12.5 L per min, and the AirPort MD8 (Sartorius, Epsom, UK) at an airflow of 50 L per min and nanopore size of 3 μm. The results of the study found that the greatest sampling efficacy was using the AirPort MD8 at 99%, followed by SKC BioSampler at 80-100% sampling efficacy. However, during the sampling period, researchers found the AirPort MD8 and BioBadge could be used without disturbing the swine population, while the SKC BioSampler was difficult to use because of its size and noise (Colenutt et al., 2016). There are numerous air samplers on the market, but what separates one from another is the ease with which it can be implemented on different farm systems and used by farm personnel. Hand-held samplers that do not require highly trained personnel have shown promising results in detecting different swine and poultry pathogens (Andersen et al., 2022; Brito et al., 2014; Colenutt et al., 2016).

However, there are limitations to this technology. Larger samplers that can handle higher amounts of the air have a higher chance of detecting the pathogen, while being difficult to use and maneuver in the farm confines. Additionally, Colenutt et al. (2016) reported virus inactivation caused by particle desiccation from prolonged sampling, and so this technology may not allow for tissue culture isolation. (Colenutt et al., 2016) also stated that liquid-based air samplers allow for the preservation of virus viability. Additionally, to collect pathogens from the air, they have to be excreted in a high enough quantity to be detected, and as such, low virus shedding might result in a false negative. Other environmental factors should also be considered, especially the airflow used in farm systems, where stagnant air flow would favor the use of air samplers and increase the possibility of pathogen detection. The number of dust particles to which possible viruses on the farm are bound could also affect the success of sampling.

Air sampling is a relatively novel sampling method to detect pathogens in different farming systems and different animal species. The main advantages of air sampling are the lack of animal contact, while the method still produces adequate samples for diagnostics. However further studies are necessary to fully understand its applications and possible negative aspects.

CONCLUSION

Non-invasive sampling methods offer a simple and relatively inexpensive means of herd monitoring. Unlike traditional sampling methods that require extensive handling of swine, thus endangering farm workers and veterinary personnel on the farm, non-invasive methods can be used without encroaching on animal welfare. Methods such as OF, NS, and USW have been successfully used for the detection of swine viral pathogens in intensive farm systems. However, negative aspects should also be considered, and the methods must be applied appropriately depending on the swine production category, age, farming conditions, disease status on the farm, etc. Many of the described sampling methods are quite novel, and further research is necessary so that they could be used routinely. Air sampling shows promising results, although shedding periods and the concentration of viruses in the air have to be considered to achieve a quality result. The use of different non-invasive sampling strategies should be considered to overcome the mentioned negative aspects of each sampling method.

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Authors' contributions

The manuscript design, literature searches, writing, and revisions of the article were conducted by all authors (DG, LJV, BM, MN, JM, BK, and VM). Additionally, all authors have reviewed and approved the final version of the manuscript.

Competing interests

The authors declare that they do not have any competing interests.

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PREGLED NEINVAZIVNIH METODA UZORKOVANJA U INTENZIVNOM SVINJARSTVU

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Kratak sadržaj

Praćenje zdravlja životinja na svinjskim farmama je od izuzetne važnosti kako bi se osigurala dobra proizvođačka praksa. Tradicionalno, aktivan i pasivan nadzor na farmama podrazumeva primenu invazivnih metoda uzorkovanja, što zahteva fiksiranje pojedinačnih jedinki i uzorkovanje. Međutim, s porastom intenziteta proizvodnje svinja, postala su neophodna alternativna sredstva za efikasno nadgledanje čitavog stada. Nenametljivo uzorkovanje pruža praktičan i ekonomičan pristup nadzoru celokupnog stada bez ugrožavanja dobrobiti životinja, istovremeno obezbeđujući odgovarajuće uzorke za testiranje. Oralni fluidi se široko koriste u praćenju zdravlja ljudi i stoke za razne virusne patogene, uključujući značajne bolesti. Nosni brisevi mogu biti napravljeni od različitih tkanina natopljenih fosfatnim puferom ili tkivnom kulturom s antibioticima i antimikoticima za uzorkovanje virusa influence svinja. Uzorkovanje kože vimena je alternativna metoda za procenu zdravstvenog stanja prasadi u leglu. Tokom rutinskih postupaka kao što su skraćivanje repa i kastracija, može se sakupiti mešavina krvi i seruma, poznata kao procesna tečnost, koja se uspešno koristi u nadgledanju stada na prisustvo virusa reproduktivnog i respiratornog sindroma svinja. Pored toga, uzorkovanje vazduha nova tehnika za otkrivanje patogena u različitim sistemima uzgoja životinja. Ova metoda pruža prednost dobijanja dijagnostičkih uzoraka bez direktnog kontakta sa životinjama. Primena neinvazivnih metoda uzorkovanja omogućava proizvođačima svinja da primene efikasne strategije nadzora, održe standarde dobrobiti životinja i pritom dobiju pouzdane dijagnostičke informacije.

Ključne reči: uzorci vazduha, neinvazivno uzorkovanje, nosni brisevi, oralni fluid, procesna tečnost, uzorci kože vimena