RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of air filters as a novel surveillance method to assess spread of airborne PRRS viruses **– NPB #19-164**

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Industry Summary:

Porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are two major respiratory pathogens of pigs that can be transmitted through the air. In this study, we evaluated the detection of both PRRSV and influenza from air filters installed in sow farms located in medium to high pig density areas to evaluate whether used air filters could work as a means to monitor the regional spread for these viruses. Filters that had been installed for 6, 8, 11 and 14 months approximately were tested for the viruses. Out of the 136 filters installed, ten (1.5%) samples corresponding to seven (5%) filters from three farms tested positive for PRRSV. During the study, PRRS outbreaks were reported in four farms, however, only one PRRSV positive filter originated from farms that had PRRS outbreaks. It is important to note that this study was conducted prior to the emergence of PRRS 144 Lineage 1C. In contrast, 65 (47.8%) filters from all seven farms tested positive for influenza, with a total of 131 samples positive (19.3%). Partial sequencing of influenza was possible in some samples and an H3N2 human-like influenza virus was detected. Our study showed that detection of PRRSV and IAV in the air filters showed potential evidence of regional airborne transmission for these viruses, but additional investigations are needed to better understand the factors that contribute to airborne transmission of these viruses. Overall, the testing of used filters for regional surveillance should be further explored since it may be beneficial for some diseases under certain circumstances. For more information contact Montse Torremorell (torr0033@umn.edu).

Key Findings:

- PRRSV was detected in a limited number of filters from sow farms located in medium to high pig density areas. However, overall incidence of infection in the farms was low which may be indicative of limited challenge to the farms.
- In contrast, influenza was readily detected in used filters from sow farms, and a partial sequence indicative of an H3N2 human-like influenza virus was characterized from the filters.
- Further investigations are needed in particular for seasons when PRRS and influenza activity is considered high.

Keywords: PRRS, influenza, filtration, surveillance, disease prevention

Scientific Abstract:

Porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are two major respiratory pathogens of pigs that can be transmitted through the air. In a prospective study, we evaluated the detection of both PRRSV and IAV from air filters installed in breeding herds of farms located in medium to high pig density areas to evaluate the use of air filters as a surveillance method to monitor the regional spread of PRRSV and IAV. Filters from either PRRSV negative or stable herds were removed at approximately 6, 8, 11 and 14 months post installation and 5 samples from each filter were tested by RT-PCR for PRRSV and IAV. A filter was considered positive if at least one sample tested positive. Samples positive for PRRSV or IAV were further analyzed with whole genome sequencing.
Out of the 136 filters installed, ten (1.5%) samples corresponding to seven (5%) filters from three farms tested positive for PRRSV. During the study, PRRS outbreaks were reported in four farms, however, only one PRRSV positive filter originated from farms that had PRRS outbreaks. In contrast, 65 (47.8%) filters from all seven farms tested positive for IAV, with a total of 131 samples positive (19.3%). Six IAV positive samples were sequenced and one sample was successfully subtyped as an H3N2 human-like influenza virus. In addition, multiple lineages were identified for the influenza internal genes from different samples. In conclusion, testing of used air filters in swine farms did not result in an enhanced surveillance method for airborne PRRSV. However, used filters for influenza surveillance should be further evaluated. Overall, detection of PRRSV and IAV in the air filters showed potential evidence of regional airborne transmission for these viruses, but additional investigations are needed to better understand the factors that contribute to airborne transmission of these viruses.

Introduction:

Among all infectious agents affecting pigs, airborne pathogens are the most costly and difficult to control (Hyslop, 1971). Porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are two major respiratory pathogens that result in significant economic losses to the pig industry in the US. Airborne transmission is considered an important route of PRRSV and IAV spread, which makes the two pathogens costly and difficult to control. Air filtration of incoming air has been widely used in swine

farms in the Midwestern U.S, and has effectively reduced the incidence of PRRSV infections in breeding herds (Dee et al., 2012). Therefore, detection of viruses in used air filters from farms offers a unique, largely unexplored, opportunity to monitor the regional spread of PRRSV and IAV. Testing of used filters may also help producers investigate the source of new virus introduction into their herds. In this study, we aimed to evaluate the use of air filters as a surveillance method to monitor the regional spread of PRRSV and IAV, and to enhance our understanding of the epidemiology and control of airborne diseases.

Objectives:

The overall goal of this proposal is to develop novel surveillance methods to enhance our understanding of PRRSV epidemiology and advance the regional control of PRRSV (porcine reproductive and respiratory syndrome virus). More specifically, we propose to:

a) Evaluate the use of air filters as a novel surveillance strategy as a means to monitor the regional spread of airborne PRRSV
b) Characterize PRRSV genomic sequences from air filters

Materials & Methods:

Farms and air filters

We selected 7 breeding herds from high pig density areas in the Midwestern United States, which were either PRRSV negative or stable (i.e produced negative pigs at weaning) at the beginning of the study. Twenty brand new air filters were installed in each farm, and five filters were removed each time at approximately 6, 8, 11 and 14 months post installation (Table 1). The filters in this study included two brands of filters of a MERV 14 or MERV 15 rating. The order of filter removal was determined at time of installation in order to obtain an even representation based on where the filters were located. We also received extra filters, which were unused but kept in storage at the company participating in the study and served as negative controls. PRRSV outbreaks in these herds during the study were recorded.

Because the incidence of PRRSV breaks in the farms enrolled in the study ended up being low, we decided to include the detection of influenza A virus (IAV) in the filters as part of the study. IAV is endemic in pigs in North America and a recognized airborne pathogen.

Table 1. Information on air filter installation and removal.

Farm	Company	No. of filters	Installation date	1st removal	2nd removal	3rd removal	4th removal
1	A	20	Jan 2020	Feb(1 mths)*	Mar (2 mths)	Apr(3mths)	May (4 mths)
2	A	20	Jan 2020	Feb(1mths)	Mar (2 mths)	Apr(3mths)	May (4 mths)
3	B	16	Jul 2019	Jan (6 mths)	Mar (8 mths)	Apr(9mths)	May(10 mths)
4	B	20	Aug 2019	Dec (4 mths)	Mar (7mths)	Jun(10mths)	Oct (14 mths)

5	C	20	Nov 2019	Dec (1 mths)	Feb (3mths)	Oct(11 mths)	Oct (11 mths)
6	C	20	Nov 2019	Dec (1 mths)	Feb (3mths)	Oct (11 mths)	Oct (11 mths)
7	C	20	Nov 2019	Dec (1 mths)	Feb (3mths)	Oct (11 mths)	Oct (11 mths)

*mths

Sample Processing

For each filter, we located five panels and identified five areas (A to E) on each panel. In each area, we cut two 2-by-2-inch squares of the unfold filter media (Figure 1)(Nirmala et la., 2021). The samples cut from B, C and D were pooled into one tube, and those from A and E were stored at -80℃. There were five pooled samples collected from each filter, with each sample consisting of six 2-by-2-inch squares. We cut all the samples from each tube into small pieces with sterile scissors separately.

Samples were cryogenically grounded with the Mill Mixer (MM400, Retsch) equipment following instructions in the manufacturer's manual and using liquid nitrogen as grinding aid. In general, the grinding jars were first filled with the sample material and a metallic ball to facilitate grinding, and once the jar was closed, it was immersed in liquid nitrogen for approximately 2-3 minutes. Then, the grinding jar was clamped into the device and the grinding conditions set to 25 Hz of frequency for 60 s. After grinding, these samples became fine particles which were suspended in 8 ml of Minimum Essential Media (MEM) and vortexed briefly.

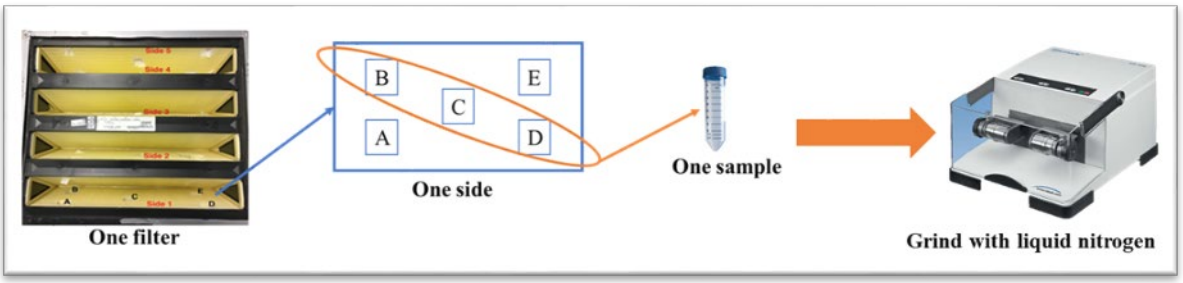


Figure 1. Steps of sample processing.

RNA Extraction

Viral RNA was extracted from all samples using TRIzol™ LS reagent (TRIzol, Invitrogen) according to the user guide instructions. In general, a volume of 250 µl suspension of the filter particles and MEM was added to 750 µl TRIzol reagent, and incubated for 5 min. A volume of 200 µl of chloroform was added to the mixture, followed by incubation for another 3 min. After centrifugation at 12,000 g for 15 min at 4℃, the upper aqueous phase was transferred into a new tube and 500 µl isopropanol were added. The mixture was then incubated for 10 min and centrifuged at 12,000 g for 15 min at 4℃. The supernatant was discarded, and the RNA pellet was washed using 1 ml of 75% ethanol, and centrifuged at 7,500 g for 5 min. The supernatant was discarded and the RNA pellet was air dried for 5–10 min. The RNA pellet was then resuspended in 30 µl of RNase-free water.

Quantitative RT-PCR

Viral RNA extracted from all samples was quantified by real time RT-PCR for PRRSV and IAV, using the AgPath-ID One Step RT-PCR kit (Applied Biosystems) following the established protocol. A volume of 7 µL RNA was used for each reaction, and the conditions were 45℃ for 10 min, 95℃ for 10 min, followed by 45 cycles of 95℃ for 15 s and 60℃ for 45 s (PRRSV), or 94℃ for 1 s and 60℃ for 30 s (IAV). These methods targeted ORF 6 of PRRSV or the matrix gene of IAV. RNA extracted from MEM and water were used as negative controls for the RNA extraction and RT-PCR reactions. Samples with Ct values < 38 were considered positive and ≥ 38 were negative. A filter was considered positive if at least one sample tested positive.

Sequencing

Samples positive for PRRSV (Ct < 38) were submitted to the University of Minnesota Veterinary Diagnostic Laboratory for next generation sequencing and genetic analysis. Viral RNA from samples positive for IAV (Ct < 38) were first amplified using SuperScript™ III One-Step RT-PCR System with Platinum™ *Taq* High Fidelity DNA Polymerase (Invitrogen) with degenerate primers (MBTuni-12 M and MBTuni-13). The PCR product was visually verified by gel electrophoresis using 1.8% agarose gel, following the established protocol. The PCR product showing clear bands was then cleaned up with Qiagen QIAquick PCR Purification Kit (QIAGEN). The purified PCR products were submitted to the Veterinary Diagnostic Laboratory of the University of Minnesota and whole genome sequenced by Illumina MiSeq platform. Clade classification for each segment was done using the automated classification tool OctoFLU[2]. FASTA files were aligned with sequences of the Influenza Research Database and reference sequences from OctoFLU using MUSCLE alignment tools.

Statistical Analysis

Spearman's correlation coefficient was used to assess the relationship between the lifetime of the filters and the proportions of positive filters.

Results:

Aim 1: Evaluate the use of air filters as a novel surveillance strategy as a means to monitor the regional spread of airborne PRRSV

A total of 136 air filters were analyzed. These filters were installed in July 2019 at the earliest, and removed successively until October 2020. Filters were cut into 680 samples for testing. Out of the 136 filters, ten (1.5%) samples corresponding to seven (5%) filters from three farms tested positive for PRRSV. In contrast, sixty five (47.8%) filters from all seven farms tested positive for IAV, with a total of 131 (19.3%) samples positive (Table 2).

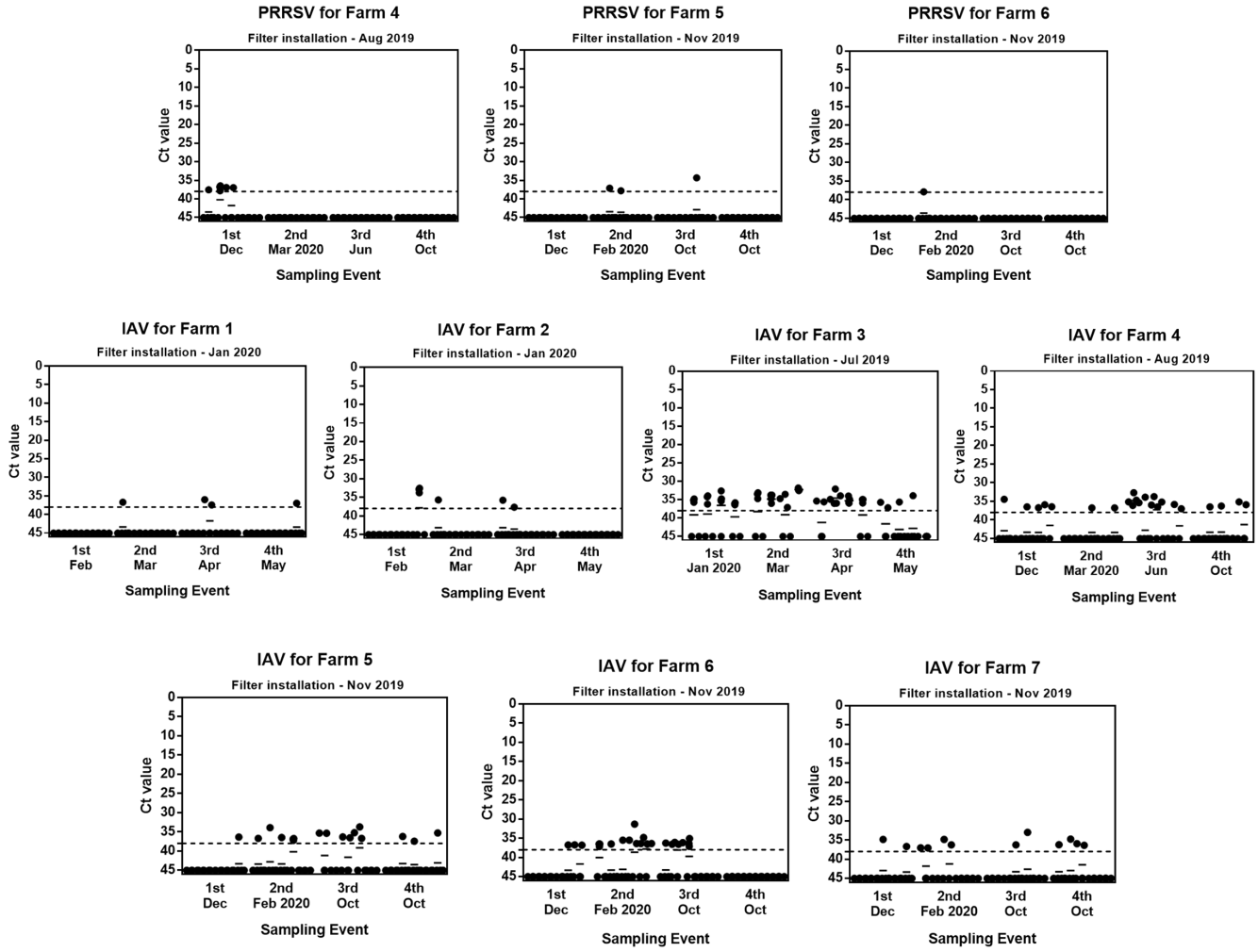
Table 2. Number (proportion) of filters and samples positive for porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) per farm.

Farm	Company	Filters Positive for PRRSV	Samples Positive for PRRSV	Filters Positive for IAV	Samples Positive for IAV
1	A	0/20	0/100	3/20 (15%)	4/100 (4%)
2	A	0/20	0/100	4/20 (20%)	6/100 (6%)
3	B	0/16	0/80	15/16 (93.8%)	48/80 (60%)
4	B	3/20 (15%)	6/100 (6%)	13/20 (65%)	22/100 (22%)
5	C	3/20 (15%)	3/100 (3%)	11/20 (55%)	17/100 (17%)
6	C	1/20 (5%)	1/100 (1%)	10/20 (50%)	23/100 (23%)
7	C	0/20	0/100	9/20 (45%)	11/100 (11%)
Total		7/136 (5.1%)	10/680 (1.5%)	65/136 (47.8%)	131/680 (19.3%)

During the study, PRRSV outbreaks were reported in four farms (Farm 1, 2, 6, 7) based on the information reported to the Morrison Swine Health Monitoring Project (MSHMP). However, only one PRRSV positive filter originated from farms that had PRRSV outbreaks (Farm 6).

The viral concentration of the positive samples for PRRSV and IAV were low overall (Figure 2). There was no evidence that the proportions of positive PRRSV or IAV filters was correlated with the lifetime of these filters (from installation to removal) ($P>0.05$).

Figure 1. Cycle threshold values of samples positive for porcine reproductive and respiratory syndrome virus (PRRSV) (top) and influenza A virus (IAV) (middle and bottom).



Aim 2. Characterize PRRSV genomic sequences from air filters

RT-PCR positive samples were submitted for sequencing. None of the PRRSV RT-PCR positive samples were successfully sequenced, likely due to the high cycle threshold values indicative on small traces of RNA material. In contrast, six IAV positive samples were sequenced and one sample from Farm 3 was successfully subtyped as an H3N2 human-like influenza virus. In most instances, only partial sequences were obtained. Multiple lineages were identified for the influenza internal genes from different samples (Table 3).

Table 3. IAV whole genome sequencing and clade classification.

Farm	Sampling Event	PB2	PB1	PA	HA	NP	NA	M	NS
2	3rd	TRIG	TRIG			**TRIG**		pdm	
3	1st	TRIG	TRIG				**N2**	**pdm**	

							2002		
3	2nd	TRIG	TRIG		**H3 2010-human like**		**N2 2002**	**pdm**	
3	2nd		Classical swine	pdm		pdm	N2 2002	TRIG	LAIV
4	3rd		TRIG				**N2 2002**	**pdm**	**TRIG**
67)	2nd		TRIG		H3 2010-human like	pdm			TRIG

Clade name in grey means the sequence length < 50% CDS of corresponding gene segment; clade name in black and bold means the sequence length ≥ 50% CDS.

Discussion:

Testing of used air filters from sow farms may represent an untapped opportunity for regional disease surveillance of airborne diseases. In this prospective study we evaluated the testing of used filters to detect both PRRS and influenza viruses. Although we were able to detect PRRSV from the used filters, the number of positive filters was low and the total amount of RNA in the filters was also low. This came to a surprise to the researchers because in a prior study, PRRSV was detected in 27% of used filters (Nirmala et al., 2021). The low detection of PRRSV in this study could be the result of various factors: a) limited regional PRRSV challenge to the farms, b) low PRRSV activity in the region, c) not enough accumulation of PRRSV genetic material in the filters to yield a detectable PCR signal, or d) the limited number of farms in the study. Although farms were selected for their location in pig dense areas, farms were conveniently selected and they may not have been challenged enough by PRRSV. There are seasonal differences in PRRS incidence which may result in lower PRRSV activity in the region and subsequent challenge to the farms. Therefore, it is possible that we were not able to detect more PRRS positive filters because we did not have enough farms in the study or there was not enough PRRSV challenge in the region. It is important to note that this study happened prior to the emergence of PRRSV 144 lineage 1C virus, which has been documented to spread rapidly between farms. Detection of PRRSV in used filters may have resulted in higher detection rates if infections had happened in a season when PRRSV activity was significant. In addition, our study also coincided with the emergence of COVID-19 pandemic which delayed the testing of filters in the laboratory due to the laboratory shut down which may have altered the quality of the samples.

In contrast, detection of IAV in filters was more prevalent. All farms had filters that tested positive and the quantity of RNA in the filters was higher. Furthermore sequencing of IAV was possible out of the filter material which was very encouraging from a disease surveillance point of view. Influenza is prevalent and endemic in pigs and that may explain why we were able to detect influenza more frequently than PRRSV. However, our methods were not able to identify the source of the IAV, whether it originated from neighboring farms or exhausted from the enrolled farm itself. Either way, results are encouraging and point at the need to further explore methods to conduct regional surveillance to better understand the regional spread of diseases through the air.

In conclusion, our study showed that detection of PRRSV and IAV in the air filters showed potential evidence of regional airborne transmission for these viruses, but additional investigations are needed to better understand the factors that contribute to airborne transmission of these viruses. Overall, the testing of used filters for regional surveillance should be further explored since it may still be beneficial for some diseases under certain circumstances.

References:

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