

American Association of Swine Veterinarians (AASV)

Project AASV Grant Application 2022

Title: Assessment of population-based sampling for detection of Influenza A virus RNA in breeding herds

INVESTIGATORS

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1. Statement of the problem

Influenza A virus in swine (IAV) is consistently within the top 3 most prevalent etiologies in respiratory disease cases within the US¹. IAV is a major contributor in the porcine respiratory disease complex along with porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*² and porcine circovirus type 2 (PCV2). IAV subtypes are classified according to the hemagglutinin (H1 and H3) and neuraminidase (N1 and N2) surface proteins. Changes in the antigenic characteristics within subtypes pose a significant concern to animal health from region-to-region based on the loss of cross-protection between different genetic clades within the H1 or H3 subtype³. IAV surveillance is necessary to allow a better understanding of the epidemiology and evolution of IAV and can be applied to policy decisions⁴. Up-to-date information on prevalence and the geographical distribution of IAV also plays an important role in ensuring that current vaccines remain relevant to the prevailing endemic strains⁵.

Active surveillance is highly significant for detecting emerging strains of IAV and assessing the threat for both swine and public health⁵. The most common specimens used for molecular testing in the United States (US) are nasal swabs, nasal wipes, and oral fluids. Recently, udder wipes have been reported with promising diagnostic sensitivity⁶⁻⁸. However, sampling options used to detect IAV vary based on the herd sensitivity of the sample type and the convenience to perform sample collection.

Family oral fluids (FOF) specimens have demonstrated the ability to be an effective population-based sample type for PRRSV RNA detection⁹. FOF allows testing more animals using fewer samples, thus offering an economic advantage over individual pig sampling¹⁰. However, studies have not yet evaluated the efficacy of FOF for the detection of IAV RNA in breeding herds where IAV prevalence is low. Also, information is lacking regarding the successive use of FOF over time as a method to monitor the presence and epidemiology of IAV in commercial herds. Therefore, this study aims to compare different sample types (FOF, udder wipes, nasal swipes) on the probability of IAV RNA detection in swine breeding herds.

2. Objectives

This project aims to:

1. Compare the probability of detection of IAV RNA between selected individual and population-based samples. The following sample types were compared: nasal wipes from sows, nasal wipes from all pigs within a litter, udder wipes, family oral fluids, and drinker wipes.
2. Simulation-based work was conducted to establish the probability of identifying IAV-positive litters using different sample sizes of nasal wipes (e.g., from one pig per litter to five pigs per litter).

3. Material and Methods

Eligibility criteria

Sow farms were selected based on the following criteria: (a) production system was willing to cooperate; (b) herd size greater than 1,000 sows; (c) modern swine production facilities located in midwestern US; (d) recent IAV diagnostic evidence (within 1 week) of IAV circulating.

Overview of study design

Weaning aged piglets (17-21 days) from three breeding herds located in the midwestern US were screened for IAV positivity using udder wipes to ensure evidence of IAV circulation. One herd tested positive and study samples were collected within 48 hours of screening test completion.

Target Population

The target population was US farms with characteristics similar to the aforementioned eligibility criteria. The study population was the single enrolled sow farm; the unit of the analysis was each sow and respective litter from the study population.

Outcome

The primary outcome was the probability of detection of IAV RNA by RT-PCR for different sample types.

Sampling scheme at the breeding farm

Samples were collected at the farm using matched sets of FOF, udder wipes, and nasal wipes from sows and all 3-week-old piglets within the respective litter. FOF sampling followed the methodology described by Almeida et al. (2021); briefly, it consisted of hanging a rope in the front of the farrowing crate, where the dam and respective suckling pigs had access to.

Sampling size justification

Fifty-seven litter-matched FOF, udder wipes, and nasal wipes were used to compare the probability of detecting IAV RNA by RT-rtPCR in a breeding farm. This sample size provided 90% confidence to demonstrate at least 50% probability of detecting IAV RNA by RT-rtPCR when the litter had at least one piglet shedding IAV. In a total, 57 FOF, 57 udder wipes, 57 nasal wipes from the dams, and individual nasal wipes from all pigs in all litters were collected. In addition, drinker samples were collected from all litters.

Diagnostic testing

All samples were collected by a study collaborator, chilled, and shipped on ice to the Veterinary Diagnostic Laboratory at the Iowa State University (ISU-VDL). All samples were tested at the ISU-VDL located in the College of Veterinary Medicine Research and Development Laboratory for IAV RNA by RT-rtPCR under the supervision of Dr. Philip C. Gauger, following standard protocols that were previously validated. The sample was considered positive when the RT- rtPCR cycle threshold Ct value (Ct) was lower than 38.

Statistical analysis and investigative procedures

Descriptive statistics were performed to report the frequency of IAV RNA detection by RT-rtPCR in each sample type using the R program v 4.1.0¹¹. Nasal wipes were used as the reference sample to determine the sensitivity of other sample types, with sows or litters being considered IAV positive with ≥ 1 positive nasal wipe sample. Kappa agreement, sensitivity, and specificity was performed in R software in EpiR package¹¹. The probability of IAV RNA detection by litter was performed based on the proportions of IAV-positive piglets from individual nasal wipes in all rooms. The probability of positive piglets was performed using logistic regression model with R studio program¹¹.

4. Results

One of the three breeding herds tested IAV RNA-positive (6/35, 17.1%) at screening utilizing udder wipes and was selected for study sample collection 48 hours later. A total of 57.9% (33/57) FOF samples tested positive, as well as 49.1% (28/57) of the udder wipe samples, and 28.1% (16/57) of the sow nasal wipe samples. A total of 15.8% (9/57) of the drinker wipe samples and 66.6% (38/57) of the piglet nasal wipes tested positive for IAV RNA (Table 1). Overall, the RT- rtPCR cycle threshold (Ct) values for positive samples ranged from 24.4 to 37.9, with FOF having the lowest medium value amongst all sample types, followed by piglet nasal wipes and udder wipes (Figure 1 and Table 1).

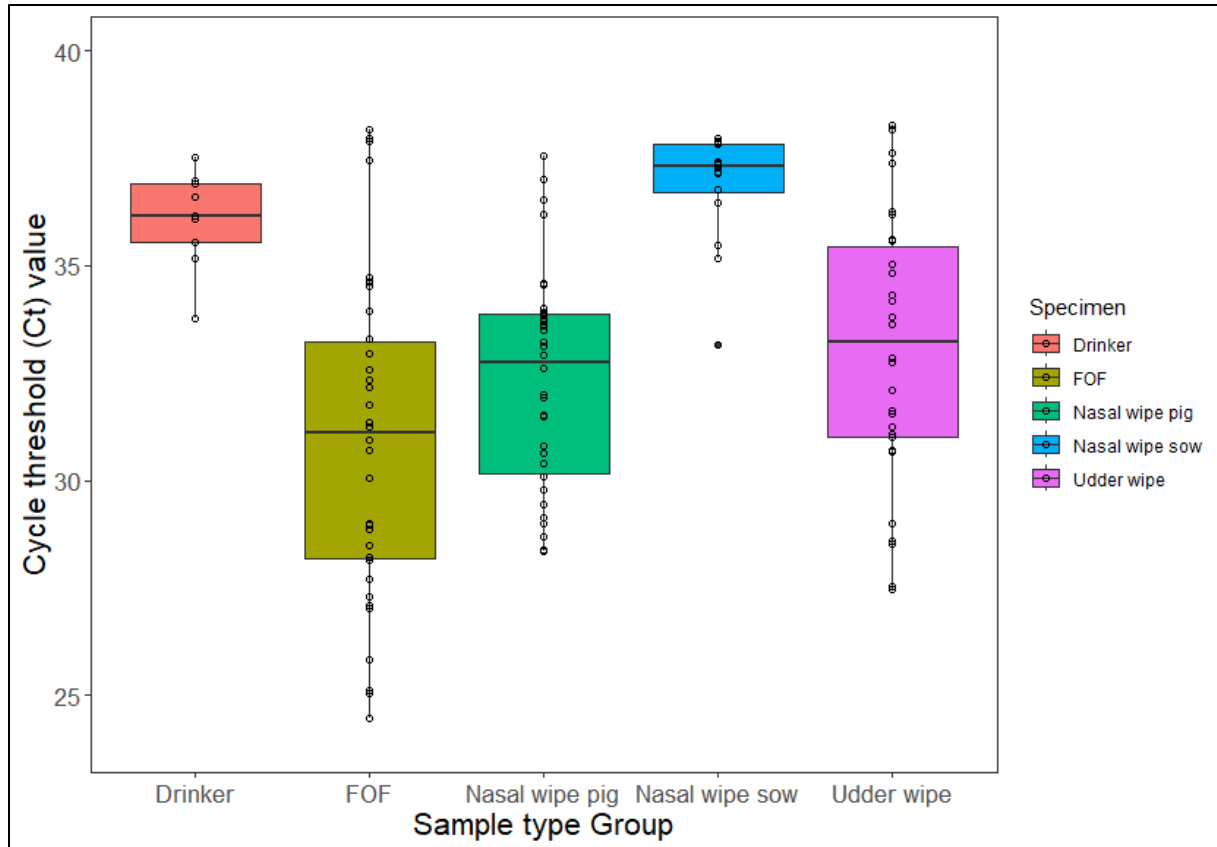


Figure 1. IAV RT-rtPCR cycle threshold (Ct) value by sample type.

There was a wide variation in percentage of positive piglets between the three rooms sampled (90.9%, 70.8%, and 9.1% for piglet nasal wipe samples in Rooms A, B, and C, respectively; Figure 2 and Table 1). This finding was in agreement with that described by Almeida et al. (2021) for PRRSV, highlighting the importance of sampling as many rooms as possible to reflect the herd status for IAV activity.

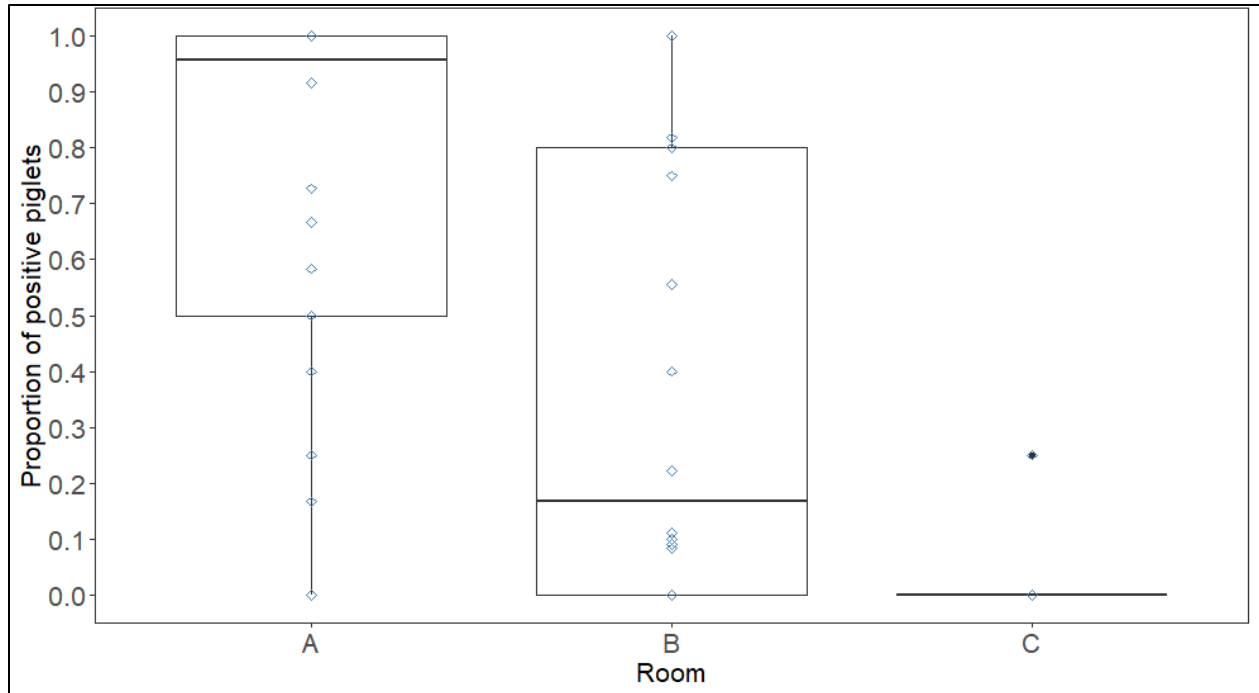


Figure 2. Proportion of IAV positive piglet nasal wipes by room.

The different sample types were compared to nasal wipe sample types to assess the agreement. The assessed agreement was based on the classification by Landis and Koch (1977), who characterized values <0 as indicating no agreement and 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1 as almost perfect agreement. Nasal wipes from piglets versus FOF presented Kappa 0.81, an almost perfect agreement, nasal wipes from piglets versus udder wipes presented Kappa 0.65, a substantial agreement. Furthermore, sow nasal wipes and drinker wipes both represented fair agreement (Kappa 0.28 and 0.24, respectively) with piglet nasal wipes (Table 2).

Table 2. Assessment of agreement between piglet nasal wipes and four other common sample types.

Comparison of diagnostics approaches	P-value	Observed agreement (sows with agreement/total sows)	Kappa (Standard error)	Sensitivity (C. I. 95%) **	Specificity (C. I. 95%) **
NW* piglets vs Family oral fluid	<0.01	0.57 (33/57)	0.81 (0.13016)	0.87 (0.72, 0.96)	1.00 (0.89, 1.00)
NW* piglets vs Udder wipes	<0.01	0.49 (28/57)	0.65 (0.124133)	0.74 (0.57, 0.87)	1.00 (0.88, 1.00)
NW* piglets vs Nasal wipe sow	<0.01	0.28 (16/57)	0.32 (0.0979)	0.42 (0.26, 0.59)	1.00 (0.82, 1.00)
NW* piglets vs drinkers	0.02	0.1578 (9/57)	0.17 (0.07415)	0.24 (0.11, 0.40)	1.00 (0.82, 1.00)

*Nasal wipes (NW) piglets were considered positive with at least one positive within a litter.

**Confidence Interval at 95%.

The probability of IAV detection by litter was performed based on the proportions of piglets that were IAV RNA positive in all rooms. The probability of detection increased as the litter prevalence increased.

In the scenario of 2 piglets in the litter testing positive for IAV using nasal wipes, FOF, udder wipes, and sow nasal wipes had an 85%, 20%, and 10% probability of testing positive respectively, for IAV, while drinker wipes presented less than a 5% probability of detection (Figure 3).

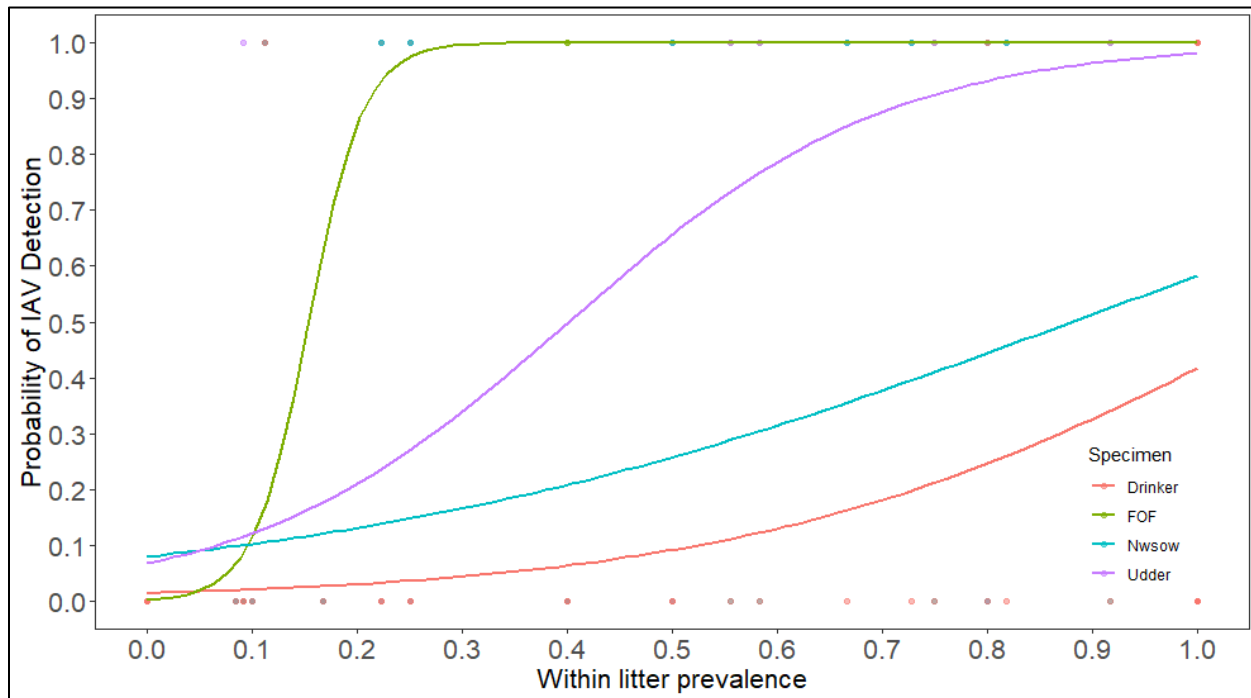


Figure 3. IAV probability of detection by within litter prevalence and sample type.

5. Discuss the most significant findings and your recommendations.

FOF and udder wipes presented higher IAV positivity compared to other sample types, using the individual piglet nasal wipes as the reference. Based on the results of this study, FOF is a resourceful alternative population-based sample type for IAV in the breeding herd, in addition to udder wipes. The room-level piglet PCR positivity ranged from 2 to 90% within the same breeding herd and same day. This emphasizes the danger of extrapolating PCR results between rooms. Instead, efforts should be made to increase coverage to multiple rooms when the purpose of sampling is to understand IAV activity within the herd.

The sample types presented a different IAV probability of detection within the litter level. Firstly, FOF presented a higher probability of detection than other sample types in this study, especially when the within-litter prevalence was lower than 50%; thus, this result suggests FOF is one resourceful option for surveillance. Secondly, udder wipes samples showed a 65% probability of detection when the within-litter prevalence was 50%, thus presenting a possible alternative for IAV surveillance. Additionally, it was possible to detect IAV from the sows through nasal wipes; samples collected from drinkers did not present a satisfactory result compared to the other sample types.

6. Describe how your findings will assist the practicing veterinarians

Population-based sampling through the use of udder wipes and family oral fluids demonstrated higher sensitivity of detection; these specimens allow for reduced costs and potential improvements in the probability of detection by increasing the number of pigs, pens, rooms, and/or sites sampled. Thus, according to these results, FOF is a great sample type to monitor IAV in the breeding herds. However,

this sample type has not been evaluated for the success of IAV sequencing, subtyping, and virus isolation at this time.

IAV can be detected at different levels of prevalence within the same farm and same age of pigs. Thus, the practicing veterinarian should consider this aspect of IAV circulation in sow farms when selecting the minimal sample size and the appropriate specimen to be used for monitoring IAV.

7. State what we can learn from this case, or the methods used to work up this case

This study showed that family oral fluids and udder wipes are promising sample types for IAV detection. Family oral fluid is an effective specimen in scenarios where IAV is expected to be present at lower prevalence within a litter. The veterinarian needs to consider how IAV can be dynamic in prevalence within the same farm and age when the sample collection is performed.

8. Itemize the take home message(s) for the audience

The take home messages for the audience were:

- A. Family oral fluids (FOF) were an effective population specimen for IAV detection in weaning-age litters. It had higher PCR positivity and lower Ct values than udder wipes and sow nasal wipes.
- B. Family oral fluid and udder wipes showed higher IAV detection, and they can be used according to the veterinarian and producer's decision and the expected within litter prevalence scenarios.
- C. Drinker wipes had low sensitivity for IAV RNA detection, even in litters of relatively high prevalence.
- D. Sample collection for IAV monitoring should be conducted in different rooms, as there may be significant differences in prevalence.

9. References

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Table 1. IAV detection by family oral fluids, udder wipes, sow nasal wipes, drinker wipes, and piglet nasal wipes at weaning age.

Room	Age	Family Oral Fluids		Udder Wipes		Sow Nasal Wipes		Drinker Wipes		Piglet Nasal Wipes ²	
		Samples positive %, ¹	Ct value average (min-max)	Samples positive %, ¹	Ct value average (min-max)	Samples positive %, ¹	Ct value average (min-max)	Samples positive %, ¹	Ct value average (min-max)	Samples positive %, ¹	Ct value average (min-max)
A	Weaning	86.3% (19/22)	29.0 (24.4-33.9)	77.2% (17/22)	32.5 (27.5-37.4)	40.9% (9/22)	36.4 (33.1-37.9)	27.2% (6/22)	35.8 (33.7-36.9)	90.9 (20/22)	31.67 (28.3-37.5)
B	Weaning	54.1% (13/24)	32.9 (25.0-37.9)	45.8% (11/24)	33.4 (27.5-36.1)	29.1% (7/24)	37.4 (37.3-37.8)	12.5% (3/24)	36.5 (35.1-37.5)	70.8 (17/24)	33.19 (28.3-37.0)
C	Weaning	9.0% (1/11)	34.5	0 (0/11)	-	0 (0/11)	-	0 (0/11)	-	9.1 (1/11)	32.92
Total		57.9 (33/57)		49.1 (28/57)		28.1 (16/57)		66.6 (38/57)		66.6 (38/57)	

¹Number of PCR-positive samples/total number of samples.

²Piglet nasal wipes were considered positive with at least one positive.