

American Association of Swine Veterinarians (AASV)

Final Report- Project AASV Grant Application 2023

Title: Probability of Influenza A virus RNA detection at different pooling levels for commonly used sample types in breeding herds

Project Type: New

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1. Statement of the problem

Influenza A virus (IAV) infection has a significant negative economic impact on pig production due to increased mortalities and decreased growth performance. A study showed that IAV infection caused economic losses as high as \$3.23 per pig. Co-infection of IAV and porcine reproductive and respiratory syndrome virus (PRRSV) or *Mycoplasma hyopneumoniae* costs \$10 per pig [1]. IAV sustains endemic infection in swine breeding herds within the suckling pig population at a low prevalence (<15%) that may impact respiratory disease in nursery pigs [2]. Therefore, IAV active surveillance is essential to understand and monitor virus activity to help swine veterinarians and producers make management, biosecurity, and disease control decisions.

The most common sample types used for IAV molecular testing in the United States (US) are oral fluids and lung tissue samples (www.fieldepi.org/sdrs-reports). Family oral fluids (FOF) are an effective population-based sample type for PRRSV RNA detection by RT-rtPCR [3]. In addition, a recent study that assessed the effect of pooling FOF on the probability of PRRSV RNA detection demonstrated that PRRSV RNA was detected at 4% prevalence using up to 5 pools of 10 FOF [4].

Recently, udder wipes have been described for IAV RNA detection with promising diagnostic sensitivity [5]. A recent study tested the effect of pooling udder wipes and showed that pooling 3 udder wipes was an appropriate alternative as a population sample for IAV RNA detection [6]. However, the effect of pooling samples on the probability of IAV detection may vary based on the sample type, pooling size, and prevalence of IAV in the population.

Population-based samples, including udder wipes (UW) and FOF, allow a reduction in diagnostic costs and improve the probability of detection (PD) by increasing the number of pigs, pens, rooms, and/or sites sampled. This approach has been successful for IAV detection in a study funded in 2022 by the American Association of Swine Veterinarian Foundation. In that study, we compared different sample types on the probability of IAV detection in swine breeding herds [7]. We hypothesized that population-based sampling (FOF, UW) provides an equivalent probability of IAV RNA detection as nasal wipes, under the same sample size. The preliminary results from that study (Table 1) showed that a total of 57.9% (33/57) FOF samples and 49.1% (28/57) udder wipes tested IAV RNA positive, with the percentage of total litters with at least one piglet positive being 66.6% (38/57) using individual nasal wipes.

Table 1: IAV RNA detection using family oral fluids, udder wipes, and nasal wipes in piglets at weaning age*.

		Family oral fluids		Udder wipes		Nasal wipes piglets	
Room	Age	Positive Samples, % ¹	Average Ct value (min-max)	Positive Samples, % ¹	Average Ct value (min-max)	Positive Samples, % ¹	Average Ct value (min-max)
A	Weaning	86.3% (19/22)	29.0 (24.4-33.9)	77.2% (17/22)	32.5 (27.5-37.4)	90.9% (20/22)	31.7 (28.7-37.5)
B	Weaning	54.1% (13/24)	32.9 (25.0-37.9)	45.8% (11/24)	33.4 (27.5-36.1)	70.8% (17/24)	33.1 (28.4-37.0)
C	Weaning	9.0% (1/11)	34.5	0 (0/11)	-	9.0% (1/11)	32.9

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

*Preliminary results from a study funded by the American Association of Swine Veterinarians Foundation (Moraes et al., 2023).

As shown in Table 1, results supported that FOF is a sensitive population-based sample type for IAV in the breeding herd. However, there was a need to further understand the effect of pooling population-based samples on the probability of IAV detection.

To achieve greater confidence in the results from population-based samples and determine the level of pooling that would demonstrate optimal detection sensitivity, a comparison of the different sample types pooled at different levels and different concentrations of the target (IAV) was needed. *Under this study we hypothesized that pooling population-based sampling increases the probability of IAV detection, assuming a fixed budget for diagnostic testing (i.e., same sample size across all sampling schemes).* The previous study using FOF significantly improved monitoring and surveillance protocols for IAV in breeding herds adding important alternatives for IAV detection, but it is still cost-prohibitive for most producers. Therefore, this study aimed to compare different sample types (family oral fluid samples, udder wipes, and nasal swipes) at different levels of pooling, on the probability of IAV detection in swine breeding herds.

2. Objectives

The objective of this project was to compare the probability of IAV RNA detection at different levels of pooling (undiluted, 1:3, 1:5, 1:10) for different sample types (FOF, Udder wipes, and Nasal wipes).

3. Material and Methods

Overview of study design

This was a prospective study targeting reverse transcription real-time PCR (RT-rtPCR)-positive samples collected from a US breeding herd. We used PCR-positive samples pooled at different levels to understand the impact on the probability of IAV RNA detection.

Outcome

The primary outcome was the probability of IAV RNA detection at different pooling levels for family oral fluid, udder wipes, and nasal wipes.

Sample size justification

Forty-five PCR-positive IAV samples (15 FOF x 15 udder wipes x 15 nasal wipes) were selected and pooled with PCR-negative samples in the following proportions: undiluted, 1:3, 1:5, 1:10. The negative samples used for the dilutions originated from a breeding herd that conducted an IAV elimination and had repeatedly tested IAV PCR negative in the same sample types. Each pooling level for each sample type was thereafter tested in replicates of six to confirm IAV status and establish cycle threshold (Ct) values, according to the method

described by Osemeke et al [4]. PCR-positive samples were categorized into Ct groups based on the preliminary experiment results (i.e., three categories: Category A - Ct value lower than 30; Category B - Ct value between 30-34; Category C - Ct value between 34-38). In summary, there were 45 positive samples, each having 4 dilution levels and 6 replicates, making 1,080 RT-rtPCR tests.

Diagnostic testing

All samples were organized and pooled by a study collaborator and tested at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) Research & Development Laboratory for IAV RNA by RT-rtPCR under the supervision of Dr. Phillip C. Gauger, following standard and previously validated protocols.

Statistical analysis and investigative procedures

Descriptive statistics were used to report the frequency of IAV RNA detection by PCR for each sample type. A linear mixed regression model was used to characterize changes in cycle threshold values with increased pooling level, using the lme4 package in the R program. Using up to six replicates improves the accuracy of the estimates from the probit regression model [4]. A probit regression model was used to estimate the probability of IAV detection for each pooling level for each sample type, using the brglm package in R statistical software [8]. In addition, data from the ISU VDL from 2018 to 2023 was analyzed to understand the cycle threshold (Ct) value distribution by Category A - Ct < 30; Category B - Ct between 30-34; Category C - Ct between 34-38 for the sample types FOF, UW, and NW.

4. Results

Undiluted samples from sample types FOF, Udder wipes, and Nasal wipes, were grouped according to comparable Ct values into Ct Categories A, B, and C (Figure 1).

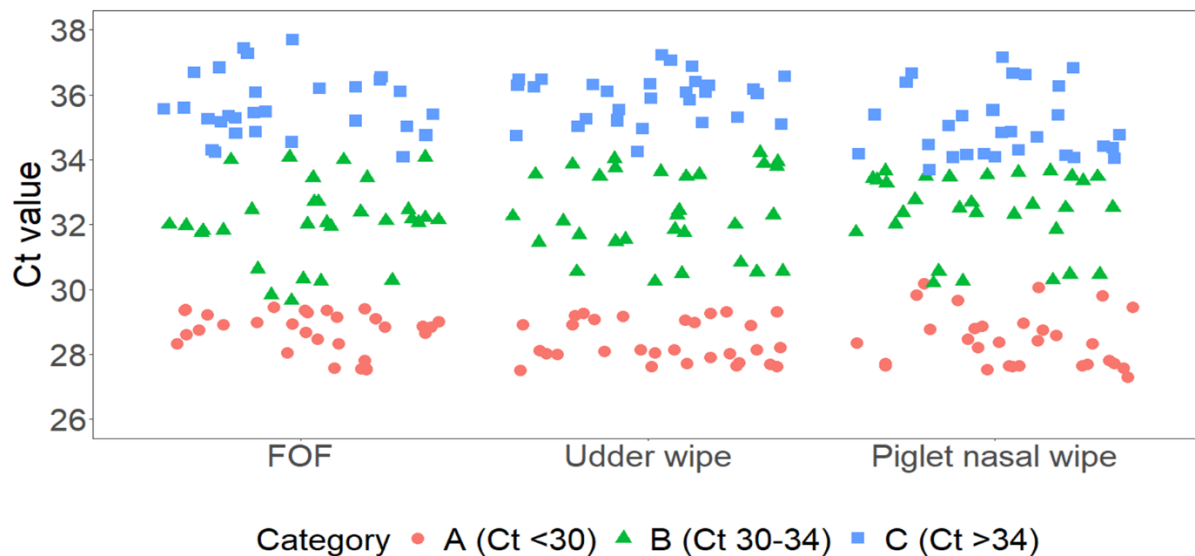


Figure 1. Undiluted samples matched based on Ct values within each Ct value Category (A, B, and C). Category A is denoted by red circles, Category B by green triangles, and Category C by blue squares.

For FOF and UW, the probability of IAV detection in Ct categories A and B did not decrease when the dilution level increased to 1/10. In category C of both sample types, the PD was observed to decrease as the dilution level increased, reaching a value of 18% at 1/10 dilution (Table 1). For NW, the PD did not decrease for all dilution levels only in category A. In addition, for NW for Category B, the dilution level was not statistically different until dilution level 1:5 (Table 2 and Figure 2).

Table 2. IAV probability detection by FOF, Udder wipes, and Nasal wipes at different dilution levels.

Dilution level	Family oral Fluids			Udder wipes			Nasal wipes		
	A Ct<30	B Ct 30-34	C Ct 34-38	A Ct<30	B Ct 30-34	C Ct 34-38	A Ct<30	B Ct 30-34	C Ct 34-38
Undiluted	0.986 ^a	0.986 ^a	0.954 ^b	0.986 ^a	0.986 ^a	0.986 ^c	0.986 ^a	0.986 ^b	0.954 ^b
1:3	0.986 ^a	0.986 ^a	0.825 ^b	0.986 ^a	0.986 ^a	0.760 ^{bc}	0.986 ^a	0.986 ^b	0.792 ^{bc}
1:5	0.986 ^a	0.986 ^a	0.435 ^a	0.986 ^a	0.986 ^a	0.565 ^b	0.986 ^a	0.727 ^{ab}	0.500 ^{ab}
1:10	0.986 ^a	0.986 ^a	0.175 ^a	0.986 ^a	0.986 ^a	0.175 ^a	0.986 ^a	0.403 ^a	0.208 ^a

^{a b c} Least-square means within a column with different letters are statistically different ($P \leq 0.05$).

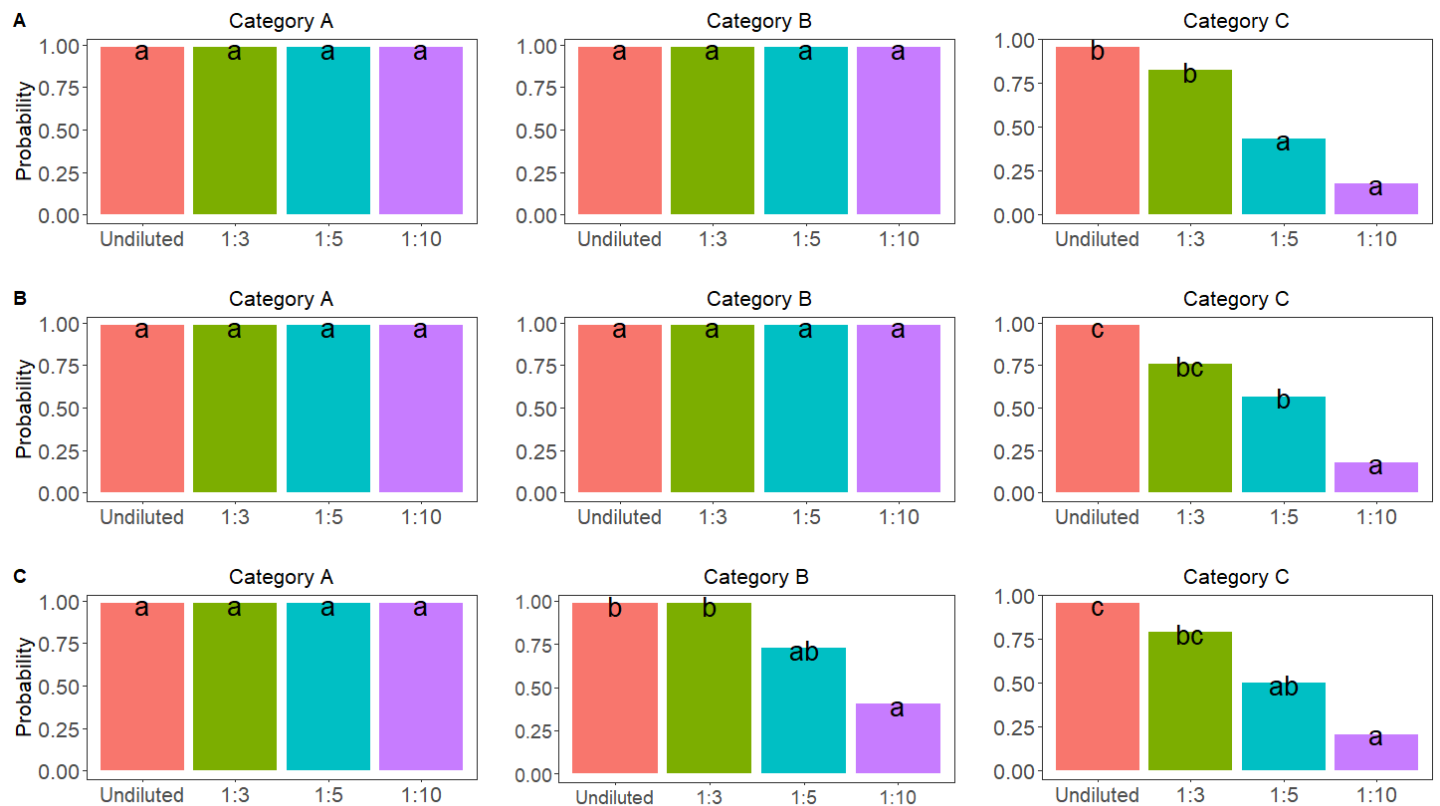


Figure 2. Probability of IAV RNA detection by Family oral fluids (A), Udder wipes (B), and Nasal wipes (C) in dilution levels (Undiluted, 1:3, 1:5 and 1:10). ^{a b c} Least-square means within a Category (A, B, or C) with different letters are statistically different ($P \leq 0.05$).

5. Discuss the most significant findings and your recommendations

This study assessed the pooling effect on the PD between different IAV surveillance samples. From data from the ISU VDL from 2018 to 2023, 80% of all the IAV RT-qPCR positive oral fluid samples from breeding herds were in Categories A and B, while 20% were in Category C. For UW, 79% of positive samples were in categories A and B, and 21% in category C. Considering NW and nasal swabs, 84% were in Categories A and B, and 16% were in Category C. These results demonstrate that about 80% of the IAV-positive samples had Ct values < 34; thus, practitioners may want to consider pooling samples to improve coverage on a fixed testing budget, testing more samples with the same budget. However, the selection of pooling size should also consider clinical history, costs, the diagnostic question, and the expected prevalence of IAV in the population.

Pooling population-based samples (i.e., udder wipes, family oral fluids) allows cost reduction and potential gains on the probability of detection by increasing the coverage of pigs, pens, rooms, and/or sites sampled. Providing these comparative probabilities on IAV detection between different sampling approaches allows veterinarians and producers to make informed decisions on IAV monitoring practices in swine populations.

6. Describe how your findings will assist the practicing veterinarians

Based on the significant findings, practicing veterinarians can leverage pooling population-based sampling, particularly pooled udder wipes and family oral fluids, to enhance the sensitivity of IAV detection in swine populations. These sample types offer potential cost savings and increased probability of detection by expanding the sampling coverage across pigs, pens, rooms, and/or sites. However, it's essential to recognize that while these samples offer advantages in detection sensitivity, their efficacy in IAV sequencing, subtyping, and virus isolation remains to be fully evaluated.

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

The study highlights the effect of dilution levels on the probability of IAV detection across different sample types. Specifically, it demonstrates how dilution affects the probability of IAV detection differently across Ct categories for each sample type. This study showed that pooled family oral fluids and udder wipes are promising sample types for IAV detection. These samples are a cost-effective strategy to enhance IAV surveillance coverage while maximizing detection probabilities. For example, while dilution did not significantly affect PD for pooled family oral fluids and udder wipes for Ct values <34, it had a notable impact on PD when the Ct value was >34. This information can guide practitioners in determining the most appropriate dilution levels for their surveillance protocols according to the prevalence in the breeding herd.

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

The take-home messages for the audience are:

- A. Family oral fluids and Udder Wipes: probability of IAV detection at Ct values < 34 did not decrease at dilutions 1:3, 1:5, and 1:10;
- B. Low prevalence farm: practitioner should consider smaller pools: 1:3 or 1:5;
- C. Practitioners should consider pooling samples to improve coverage and reduce cost.

9. References

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