American Association of Swine Veterinarians (AASV)

Interim 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 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 vary based on the sample type and pooling size.

Population-based samples, including udder wipes (UW) and FOF, allow a reduction in diagnostic costs and improve the probability of detection 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

[1]Number 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 supportted 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

This project aims to:

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 real-time, reverse transcription 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 Influenza A virus 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. (2022) [4]. PCR-positive samples were categorized into Ct groups based on the results of the preliminary experiment (i.e., three categories: Category A - Ct value lower than 30; Category B - Ct value between 30-35; Category C – Ct value between 35-38). 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 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 PD for each pooling level for each sample type, using the brglm package in R statistical software [8].

4. Preliminary Results

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 (Table 1).

Table 1. IAV probability detection by FOF, udder wipes, nasal wipes in 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 ($P \leq 0.05$).

*Preliminary results from Table 1 were submitted and will be presented in the Research Topics in the AASV Meeting (Moraes et al., 2024).

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 Iowa State University Veterinary Diagnostic Laboratory from 2018 to 2022, 77% of all the IAV RT-qPCR positive oral fluid samples from breeding herds were in Categories A and B, while 23% were in Category C. For UW, 67% of positive samples were in categories A and B, and 33% in category C. Considering NW and nasal swabs, 75% were in Categories A and B, and 25% in Category C. These results suggest that about 70% to 80% of the time, the Ct values of all three sample types are within Categories A and B; practitioners should, therefore consider pooling samples to improve coverage on a fixed testing budget, testing more samples with the same budget.

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, especially in case of low IAV prevalence (< 15%) in breeding herds.

6. Project timeline

The project is under the expected timeline. The data analysis and the final report are expected to be completed before February 28, 2024. In addition, this study has been following the same design and protocol that was proposed. No modifications to the project are expected.

References

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