American Association of Swine Veterinarians (AASV)

Interim Report- Project AASV Grant Application 2024

Title: Assessing the effect of pooling commonly used samples on the probability of Influenza A virus sequencing and virus isolation

Project Type: New

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1. Statement of the problem

Monitoring the Influenza A virus (IAV) in swine populations is essential for understanding its transmission dynamics in real-world field conditions. A study funded by the AASV Foundation (1) showed that family oral fluids (FOF) are an effective sample type for IAV detection using reverse transcription real-time PCR (RT-rtPCR). Similarly, recent research investigated using udder wipes (UW) for IAV RNA detection (2), with pools of 3 UW samples demonstrated to have high diagnostic sensitivity and, therefore, effective for surveillance purposes (3).

Population-based samples, including 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. Additionally, a study (4) evaluated different pooling levels (undiluted, 1:3, 1:5, 1:10) for FOF, UW, and nasal wipes, presenting that for FOF and UW, the probability of IAV detection in samples with Ct values lower than 34 did not decrease when the dilution level increased from undiluted to 1/10.

Pooling has also been evaluated in other swine viral pathogens. For instance, a study on PRRSV demonstrated that PRRSV RNA was detected at 4% prevalence using up to 5 pools of 10 FOF. (5). However, while pooling can improve herd-level detection sensitivity and reduce costs, its impact on IAV detection, sequencing, and virus isolation (VI) may vary depending on the sample type and degree of pooling.

The previous two studies (1,4) using FOF significantly improved monitoring and surveillance protocols for IAV in breeding herds, adding important alternatives for IAV detection. The previous two studies also assessed the probability of IAV RNA detection at different pooling levels for commonly used sample types in breeding herds. Results demonstrated the sensitivity did not significantly decrease for FOF and UW up to a Ct value of 34. However, the effect of these sample types and pooling on sequencing and virus isolation success is still unknown. Therefore, This study aims to evaluate the success of IAV sequencing and virus isolation at various cycle threshold levels using pooled swine samples, including undiluted, 1:3, and 1:5 dilutions of FOF, udder wipes, and nasal wipes.

2. Objectives

The objective of this study is to compare the success of IAV sequencing and virus isolation at various cycle threshold levels using pooled swine samples, including undiluted, 1:3, and 1:5 dilutions of FOF, udder wipes, and nasal wipes.

3. Material and Methods

Overview of study design

This prospective study targeted different cycle threshold values from IAV RT-rtPCR (PCR) positive pooled diagnostic samples collected from US breeding herds to evaluate the success of sequencing and virus isolation.

Outcome

The primary outcomes of interest are the success of IAV sequencing (total or partial HA gene) and virus isolation at different Ct values from pooled FOF, UW, and nasal wipes

Sample size justification

A total of 108 IAV PCR-positive samples (36 FOF, 36 UW, 36 nasal wipes) were selected and grouped based on the cycle threshold (Ct) values in two categories: Category A (Ct between 26-30), and Category B (Ct of 30-34) (Moraes et al. 2024). Each of these samples were tested by PCR undiluted, and in pools of 1:3, and 1:5 (Figure 1). For sequencing and virus isolation, six replicates of each pooling level for each Ct category and each sample type were tested according to the method described by Osemeke et al. (2022).

Diagnostic testing

A study collaborator organized and pooled all samples and tested them in a National Animal Health Laboratory Network (NAHLN) level 1-accredited Veterinary Diagnostic Laboratory (VDL) for IAV sequencing and virus isolation, following standard and previously validated protocols.

Statistical analysis and investigative procedures

A logistic regression model was used to calculate the probability of obtaining IAV sequences (full or partial) given the pooling level, Ct category, and sample type (6). The analysis was performed using the R program. This study classified an incomplete HA sequence as partial if the HA1 portion of the HA gene could be generated. Sequence results were reported as negative if the HA1 gene could not be sequenced. Pooling levels were evaluated based on positive or negative for VI.

4. Results

Descriptive analysis was completed as expected (Table 1).:

- For FOF, 14 of the 18 samples had complete sequences in category A. In category B, 2 out of 18 samples had complete sequences at the undiluted level.
- For UW, 7 of 18 samples had complete sequences in category A. In category B, 2 of 18 samples had complete sequences at the undiluted level.
- For nasal wipes, 11 of 18 samples had complete sequences in category A. In Category B, there were two complete sequences at the undiluted level.
- There was no statistical difference (P-value > 0.05) when assessing the IAV sequencing success by sample type at the dilution levels.

Table 1. IAV HA gene full and partial sequencing by family oral fluids, udder wipes, and nasal wipes.

Dilution level	Family Oral Fluids				Udder Wipes				Nasal Wipes			
	A Ct 26-30 %, n[1]		B Ct 30-34 %, n[1]		A Ct 26-30 %, n[1]		B Ct 30-34 %, n[1]		A Ct 26-30 %, n[1]		B Ct 30-34 %, n[1]	
	Full	Partial	Full	Partial	Full	Partial	Full	Partial	Full	Partial	Full	Partial
Undiluted	0.79 (5/6)	0 (0/6)	0.35 (2/6)	0.50 (3/6)	0.35 (2/6)	0.65 (4/6)	0 (0/6)	0.50 (3/6)	0.79 (5/6)	0 (0/6)	0.20 (1/6)	0 (0/6)
1:3	0.79 (5/6)	0 (0/6)	0 (0/6)	0.35 (2/6)	0.35 (2/6)	0.65 (4/6)	0 (0/6)	0 (0/6)	0.65 (4/6)	0.20 (1/6)	0.20 (1/6)	0 (0/6)
1:5	0.65 (4/6)	0 (0/6)	0 (0/6)	0.20 (1/6)	0.50 (3/6)	0.50 (3/6)	0 (0/6)	0 (0/6)	0.35 (2/6)	0.50 (3/6)	0 (0/6)	0 (0/6)
Total sequences %, n[1]	0.78 (14/18)	0 (0/18)	0.11 (2/18)	0.33 (6/18)	0.39 (7/18)	0.61 (11/18)	0 (0/18)	0.17 (3/18)	0.61 (11/18)	0.22 (4/18)	0.11 (2/18)	0 (0/18)

[1]Number of sequences/total samples tested by each dilution level and sample type.

*Preliminary results from Table 1 were submitted and will be presented in the Research Topics in the AASV Meeting 2025 (7).

5. Discuss the most significant findings and your recommendations.

Full sequences were recovered from FOF and NW on both Ct categories, while for UW, full sequences were only recovered when Ct was lower than 30. Overall, the success rate in obtaining full sequences in Category A was consistent until the 1:3 dilution. The success rate for obtaining sequences was much lower when the Ct value was between 30-and 34 across all sample types, indicating that pooling should be used cautiously in such scenarios. Comparison of these pooled sample types is critical for guiding practitioners and veterinarians in selecting the most effective sampling approach by the Ct value for successful IAV sequencing in breeding herds.

6. Project timeline

The project is well within the expected timeline. The data analysis and the final report are expected to be completed by February 28, 2025. In addition, this study has followed the proposed design and protocol. No modifications to the project are expected.

References

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