American Association of Swine Veterinarians (AASV)

Interim Report- Project AASV Grant Application 2024

Title: Further characterization of PRRSV diversity and other pathogens in Live Virus Inoculation (LVI) material used in breeding herd stabilization programs.

Project type: New

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1. Statement of the problem

PRRS (Porcine Reproductive and Respiratory Syndrome) is a devastating viral disease that wreaks havoc on the swine industry, causing respiratory distress in growing pigs and reproductive failure in breeding animals[1], [2]. PRRSV belongs to two distinct species: PRRSV-1 (*Betaarterivirus europensis*), also known as European type, and PRRSV-2 (*Betaarterivirus americense)* also known as North American type. The causative agent, PRRSV, is widespread across the United States, inflicting an estimated $1.2 billion in annual losses[3]. Producers often turn to commercially available modified live virus (MLV) vaccines [4] and live viral inoculum (LVI) for herd exposures to control PRRS outbreaks. MLV vaccines are produced under controlled conditions and their contents are well-characterized. The same cannot be said for LVI, which presents significant risks as it could contain uncharacterized PRRSV strains or other pathogens without proper inactivation or attenuation. This study aimed to characterize the practices implemented for LVI preparation, LVI expected viral loads, number of PRRSV strains present, and identify the potential presence of other bacterial and viral agents in LVI samples.

2. Objectives

This study aims to characterize the genetic diversity of PRRSV and investigate the potential presence of additional pathogens of interest in LVI samples used in swine production.

3. Materials and methods

Overview of the study design

This is an observational study that focuses on characterizing PRRSV strains and the genetic profiles of any additional pathogens that are present in live virus inoculation (LVI) samples

utilized for PRRSV exposure in U.S. breeding herds. Serum samples collected from breeding herds that experienced a PRRSV outbreak and subsequently used in LVI as a method of herd exposure to control PRRS outbreaks were submitted (and are in the process of being submitted) for PRRSV quantitation using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and next-generation sequencing (NGS) with a pathogen discovery approach. This approach assesses the viral load of PRRSV in the samples, identify PRRSV genetic variability, and detect any other pathogens in the LVI samples, providing a comprehensive profile of the microbial landscape.

Outcome

The project aims to deliver a comprehensive characterization of PRRSV in LVI samples by quantifying viral load, identifying the presence of one or more PRRSV strains, and detecting any additional swine pathogens using next-generation sequencing (NGS) data. This approach will yield a detailed genetic profile of PRRSV and any co-occurring pathogens, enhancing our understanding of pathogen diversity within these samples.

Sample size justification

A total of fifty LVI samples, derived from either serum or lung tissue, will undergo RT-qPCR to quantify viral particles present. Following quantification, these samples will be submitted for next-generation sequencing to identify novel or uncharacterized pathogens within the samples.

4. Preliminary results

Forty (n=40) LVI serum samples were already included in this study. Samples were collected, primarily from multiple piglet donors (70% [n= 28]; median = 5 donors, max =49 donors). LVI was administered to the entire breeding herd. Saline (52%, n= 21) was the most common diluent, followed by PBS (42.5%, n= 17). Antibiotics were not reported as added to LVI in 77.5% (n= 31) of cases. LVI was used alone in half of the cases and combined with MLV or killed PRRS vaccines in the rest. Intramuscular injection was the sole exposure method used by study participating herds, with single administration in 75% (n=30) of cases, twice in 20% (n=8), and three times in 2.5% (n=1). PRRSV concentration administered at the time of injection ranged from 49 to 88,350,183 particles/ml (median: 26,865 particles/ml and mean: 5,175,315 particles/ml; Figure 1). In 15.38% (n=6) of LVI samples more than one unique PRRS virus was recovered. Preliminary results also indicate presence of other pathogens like *Salmonella enterica, Escherichia coli, Streptococcus suis,* and *Staphylococcus aureus* those still needed final confirmation.

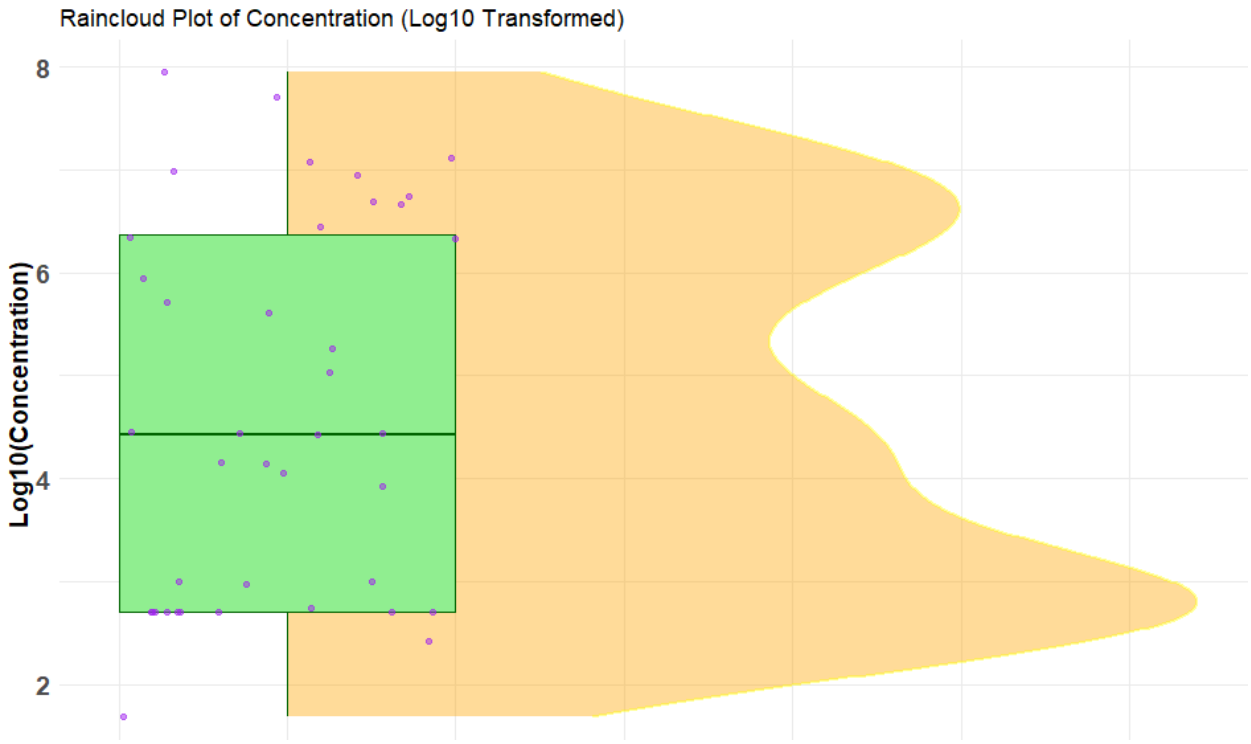

Figure 1: Raincloud plot for the expected number of PRRSV viral particles per ml of live virus inoculum. Dots represent individual results. The box plot shows its quantile, and shaded are the distribution of individual LVI samples.

5. Project timeline

The project is progressing according to the anticipated timeline, with data analysis and the final report scheduled for completion by 05/31/2025. Furthermore, the study has adhered to the originally proposed design and protocol, and no modifications to the project are anticipated.

Reference

[1] D. C. L. Linhares, J. P. Cano, M. Torremorell, and R. B. Morrison, "Comparison of time to PRRSv-stability and production losses between two exposure programs to control PRRSv in sow herds," *Prev Vet Med*, vol. 116, no. 1–2, pp. 111–119, Sep. 2014, doi: 10.1016/j.prevetmed.2014.05.010.

[2] Jeffrey J. Zimmerman *et al.*, "Diseases of Swine, 11th Edition," 2019.

[3] Osemeke OH, Silva GS, Corzo C, Kikuti M, and Xiaomei Y, "Updating the productivity and economic cost of PRRSV in the US," 2024.

[4] C. Chae, "Commercial prrs modified-live virus vaccines," Feb. 01, 2021, *MDPI*. doi: 10.3390/vaccines9020185.