AASV Foundation Research Report – Interim Report

Title: Generation of antisera against six commercial PRRSV modified live virus vaccines to evaluate their *in vitro* cross-neutralization against genetically diverse field and laboratory isolates of PRRSV

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Report

1. Statement of the problem

Porcine reproductive and respiratory syndrome (PRRS), characterized by reproductive failure in breeding females and respiratory distress in pigs of all ages, is still an economically important disease for the swine industry. In the U.S., the circulating PRRS virus (PRRSV) is mainly PRRSV-2 (North American type) which has the high degree of genetic and antigenic variability. Currently, in the U.S., six commercial modified live virus (MLV) vaccines derived from PRRSV-2 strains are available to combat against PRRS. These include Ingelvac PRRS MLV and Ingelvac PRRS ATP from Boehringer Ingelheim, Fostera PRRS from Zoetis, Prime Pac PRRS RR from Merck, Prevacent PRRS from Elanco, and PRRSGard from Pharmgate. Protective efficacy of these PRRSV vaccines was only evaluated using a few PRRSV strains as challenge viruses, and the protective efficacy of these vaccines against most PRRSV field isolates remains unknown. Consequently, when PRRSV is isolated from a farm, guidance on which vaccine(s) to use against the particular isolate is lacking. Currently, swine veterinarians often request ORF5 sequencing on PRRSV field strains; subsequently, the ORF5 sequence of the field strain is compared to the sequences of vaccine strains, and the most closely related one is frequently recommended. However, there are no validated criteria to determine which percentage of ORF5 nucleotide identity will provide protection. In addition, ORF5 (603 nucleotides) only covers ~4% of the entire PRRSV genome and protective efficacy may be related to other genes besides ORF5. Therefore, other data, in addition to ORF5 sequence comparison, should be explored to help select appropriate PRRSV vaccines for use.

The measurement of protective immunity induced by PRRSV vaccines may be a good approach to evaluating protective efficacy and vaccine selection. What parameters can reflect PRRSV protective immunity? Numerous studies have indicated that PRRSV neutralizing antibodies (NAb) are a *bona fide* parameter of PRRSV protective immunity and serum NAb titer correlates with protection. Another important component of PRRSV

protective immunity is cell-mediated immunity. However, cell-mediated immunity against PRRSV has overall not been well studied due to a lack of reagents to characterize T cell responses in swine. So far, interferon-gamma ELISPOT assay that measures the number of helper T cells and/or cytotoxic T cells producing IFN-gamma has demonstrated promise to study PRRSV-specific T cell responses. However, interferon-gamma ELISPOT assay is technically challenging to optimize and it is expensive and laborious to perform. In contrast, it is a routine technique to conduct neutralizing antibody assay in a diagnostic laboratory. Therefore, measuring PRRSV neutralizing antibody is a good start to assess PRRSV protective immunity.

It is clear that neutralizing antibody plays an important role in combating PRRSV. The key is whether a wild-type PRRSV infection or vaccination can induce high titers of PRRSV NAb and whether the NAb is cross-protective against genetically diverse PRRSV strains. Currently, little information is available about the capability of each vaccine antisera to neutralize genetically diverse PRRSV field isolates. In this study, we propose to vaccinate pigs to generate antisera against six commercial PRRSV-2 live vaccines currently available in the U.S. Subsequently, *in vitro* cross-neutralization assay will be conducted to evaluate the capability of each vaccine antisera to neutralize various PRRSV-2 laboratory and field isolates.

2. Objectives

2.1 Generate antisera against six commercial PRRSV-2 MLV vaccines by experimentally vaccinating pigs.

2.2 Conduct *in vitro* cross-neutralization assay to determine the neutralizing antibody titers of each vaccine antisera against PRRSV-2 field isolates representing different genetic lineages and sublineages.

3. Current status of this project

We initially planned to start the proposed animal work in October to November of 2021. However, as you know, the PRRSV 1-4-4 L1C variant strain emerged in the U.S in late 2020 and has caused significant losses to the U.S. swine industry. People were anxious to know if this strain is truly highly virulent in pigs under the controlled experimental condition. In August 2021, I obtained funding from Iowa Pork Producers Association to characterize the virulence and transmissibility of the newly emerged PRRSV 1-4-4 L1C variant strain in experimentally inoculated pigs in comparison with other PRRSV strains. Due to the urgency of that study, we used the reserved animal facility for that PRRSV study. The preliminary data from that study have been shared with the swine industry and a manuscript in preparation. Therefore, we postponed the AASV project with regards to generating vaccine antisera for *in vitro* evaluation.

Recently, I have discussed with the Iowa State University Animal Facility manager about the schedules of our animal study. Since one animal facility (the Building 29) at ISU is old and is not allowed to be used during June to September due to hot temperature. I was told that the best window to conduct the pig study is September to November of 2022. I have reserved the animal facility from September to November of 2022 to conduct the animal work described in the AASV 2021 and AASV 2022 proposals. So, the new timeline is to

complete the animal work in November 2022 and complete the *in vitro* neutralization work in 2023. Hence, we will request one-year extension to this project with the new completion date of June 30, 2023. Thank you for your understanding.