AASV Foundation
Final Report

Development of a diagnostic platform for *in situ* detection and subtyping of PRRSv within histological lesions

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Statement of the problem

Over the last 20 years, continuous emergence of new outbreaks associated with Porcine reproductive and respiratory syndrome virus (PRRSv) infection has challenged the swine industry. Sequencing of the ORF5 gene, which encodes a major envelope surface glycoprotein (GP5), demonstrated high genetic diversity and has been widely used to define new PRRSv introductions in a production system. Different cutoffs have been established to define these new introductions based on the percentage differences (e.i. 5%) of the ORF5 nucleotide sequences compared to the "resident" virus. The definition of these cutoffs is fairly subjective and has not always reflected the clinical and pathological characteristics observed by practitioners in the field.

Positive PCR often represents a pool of various PRRSv strains within a single sample. But during the sequencing process only sequences from one PRRSv strain are detected probably due to amplification biases. This scenario generates misleading results which may drive inaccurate interpretation and decisions for controlling the disease. The development of diagnostic tools for *in situ* detection and genotyping PRRSv based on the OFR5 sequence would be critical to genetically characterize virus strains present within lung lesions which will be directly correlated with the clinical outbreak.

Value of the research to swine veterinarians

The proposed research will benefit the swine industry by offering a rapid diagnostic tool to genetically characterize PRRSv strain in association with histopathological lesions. This platform will help to better define introduction of a new PRRSv by specifically detecting RNA sequences present within the lesions. This approach will also assess whether the sequencing method currently used is detecting the PRRSv strain that is actually in the lesions, and consequently causing the clinical disease.

Objectives

1. **Collect**, **process** and **evaluate** histological lesions in animals experimentally-infected to a reference PRRSv strain and a high virulent strain
2. **Develop** and **validate** a novel RNA-ISH for *in situ* detection and genotyping PRRSv strains in histological lesions.

Materials and methods

Experimental infection

The present research project is being performed in conjunction with research project funded by the Swine Disease Eradication Center at the University of Minnesota. Formalin-Fixed Paraffin-Embedded (FFPE) tissue specimens were obtained from experimentally-infected animals (n=8). Tissue distribution of a high pathogenic PRRSV strain (HP) to a well characterized PRRSV strain (MN1-8-4). The MN184 and HP groups were inoculated intramuscularly with 1 mL/5 x 10^3 TCID50/ml.

On day 14 post-inoculation, pigs were euthanized and samples were collected from the brain and lung of each pig for (i) PCR, (ii) histopathology investigation in order to assess the severity of lesions and (iii) the distribution of the replicating virus by ISH. Two pathologist blinded to the group's classification graded histological lesions and ISH staining, according with the following:

- Lung
    - Histological score was determined by collecting one sample from the cranial, medial, and caudal lobes that were each scored as follows 0=no lesions, 1=mild interstitial pneumonia (IP), 2=moderate IP and 3=marked IP.
    - ISH score: 0=negative, 1=focal hybridization signal, 2=multifocal hybridization signals and 3=diffuse hybridization signals.

The hybridization signals observed in the ISH staining represents metabolically-active virus characterized by the detection of viral ORF mRNA.

ISH development

Probes targeting specific regions of PRRSv genome were developed specifically focused on ORF5 RNA locus. For positive controls, probe from a well-characterized PRRSv strain were designed from animals experimentally infected that have been positive for on IHC.

Results

Experimental infection

Pigs in the negative control group did not have elevated body temperature nor show any clinical sign of respiratory and remained PRRSV negative throughout the study. Three pigs (25%) showed signs of dyspnea. Coughing was observed starting six days post-inoculation (DPI).

Among the pigs from the MN184 group, two had no lesions of interstitial pneumoniae (IP), two had mild lesions, four had moderate lesions and three had severe lesions of IP.

MN 1-8-4 strain

PCR Ct value	Histo lesions (score)	ISH score
Neg	Mild interstitial pneumonia (+1)	+1
Neg	No lesions (0)	0
28.1	Mild interstitial pneumonia (+1)	+1
18.5	Moderate interstitial pneumonia (+2)	+2
16.6	Moderate interstitial pneumonia (+2)	+2
16.7	Moderate interstitial pneumonia (+2)	+3
19.1	Moderate interstitial pneumonia (+1)	+2
16.4	Moderate interstitial pneumonia (+2)	+2

Table 1- Comparison among PCR Ct values, histological lesions and ISH scores in the lung of experimentally infected animals.

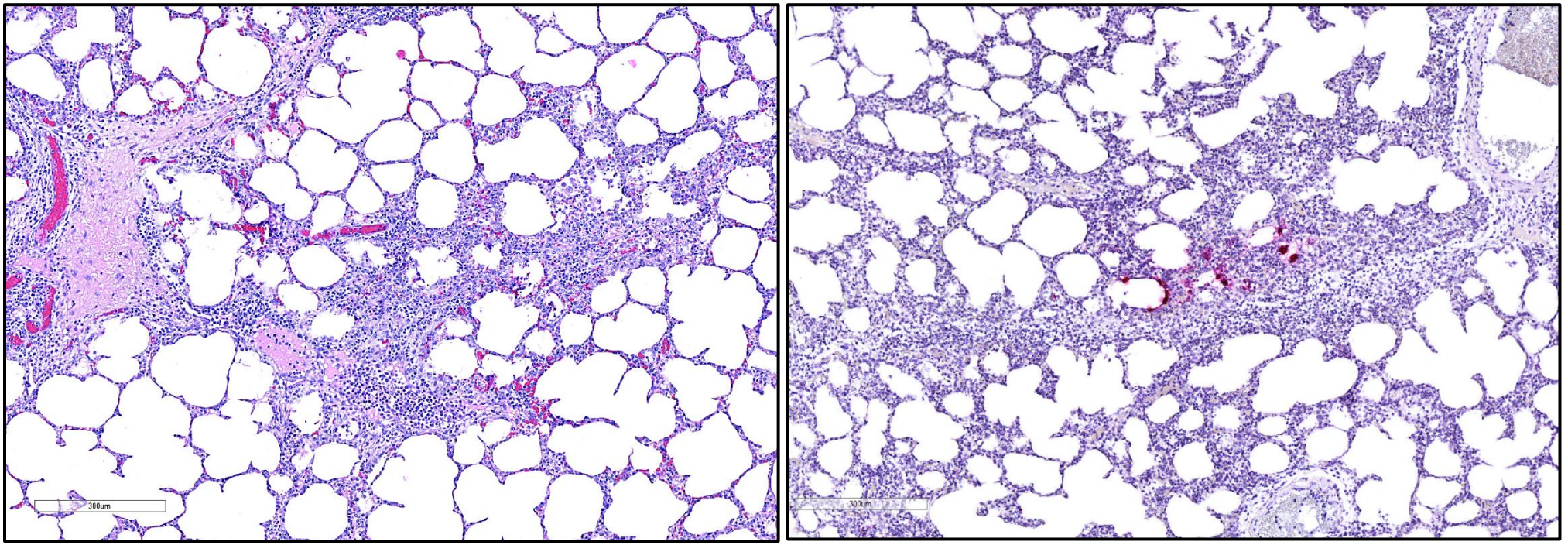

Figure 2 – Lung, A. HE staining, mild interstitial pneumonia represented by multifocal thickening of the interalveolar septa due to the lymphohistiocytic inflammatory infiltration. B. ISH staining, focal hybridization signals characterized by low levels of PRRSv replication (ORF mRNA).

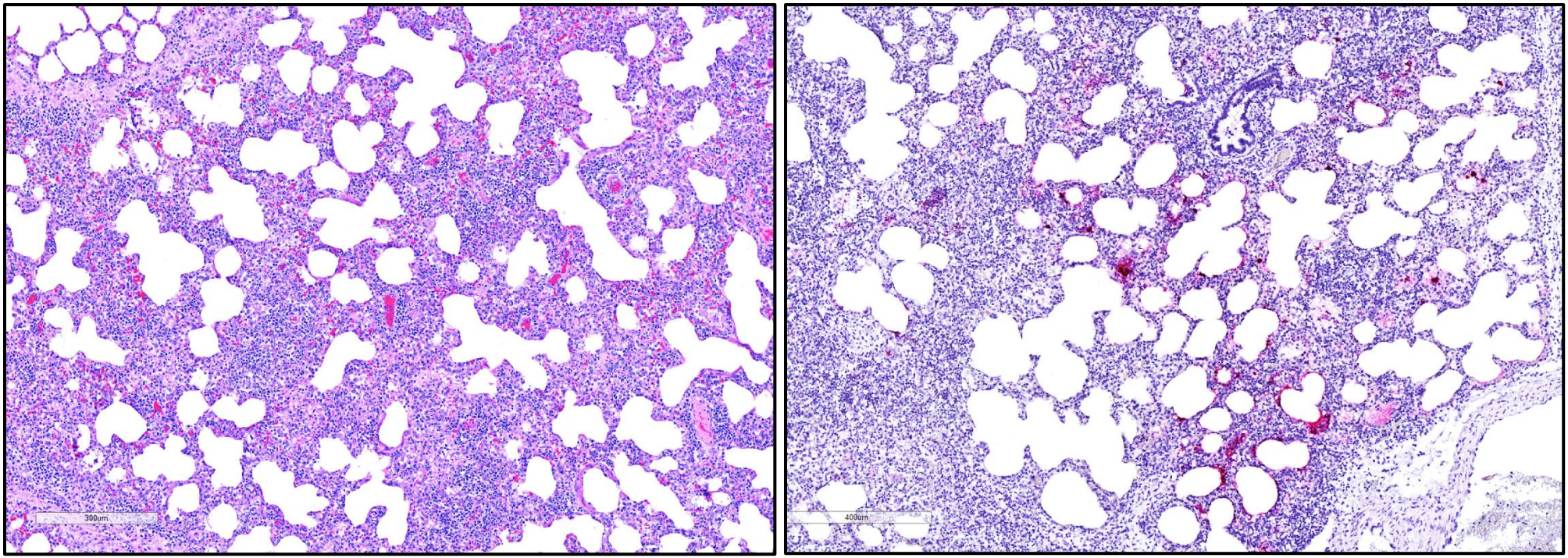

Figure 3 – Lung, A. HE staining, moderate interstitial pneumonia represented by multifocal to coalescent thickening of the interalveolar septa due to the lymphohistiocytic inflammatory infiltration and occasional necrosis of alveolar macrophages and type II pneumocytes. B. ISH staining, multifocal hybridization signals characterized by moderate levels of PRRSv replication (ORF mRNA).

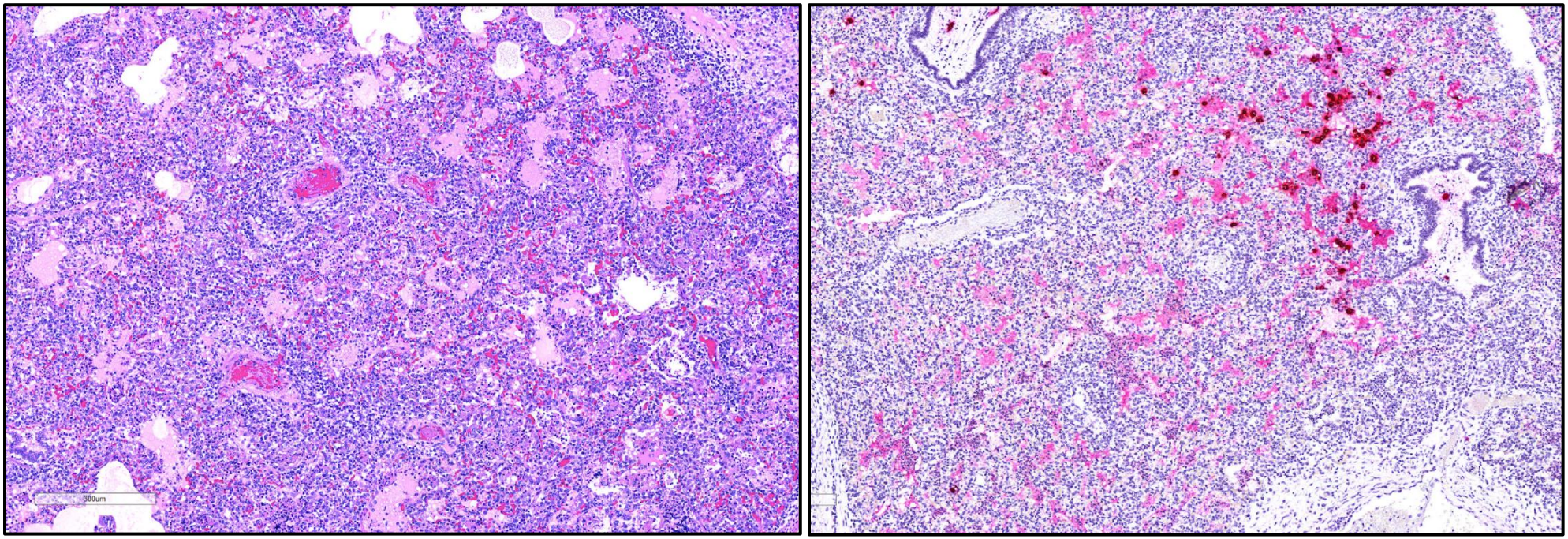

Figure 4 – Lung, A. HE staining, moderate interstitial pneumonia represented by diffuse thickening of the interalveolar septa due to edema, the lymphohistiocytic inflammatory infiltration and marked necrosis of alveolar macrophages and type II pneumocytes. B. ISH staining, diffuse hybridization signals characterized by high levels of PRRSv replication (ORF mRNA).

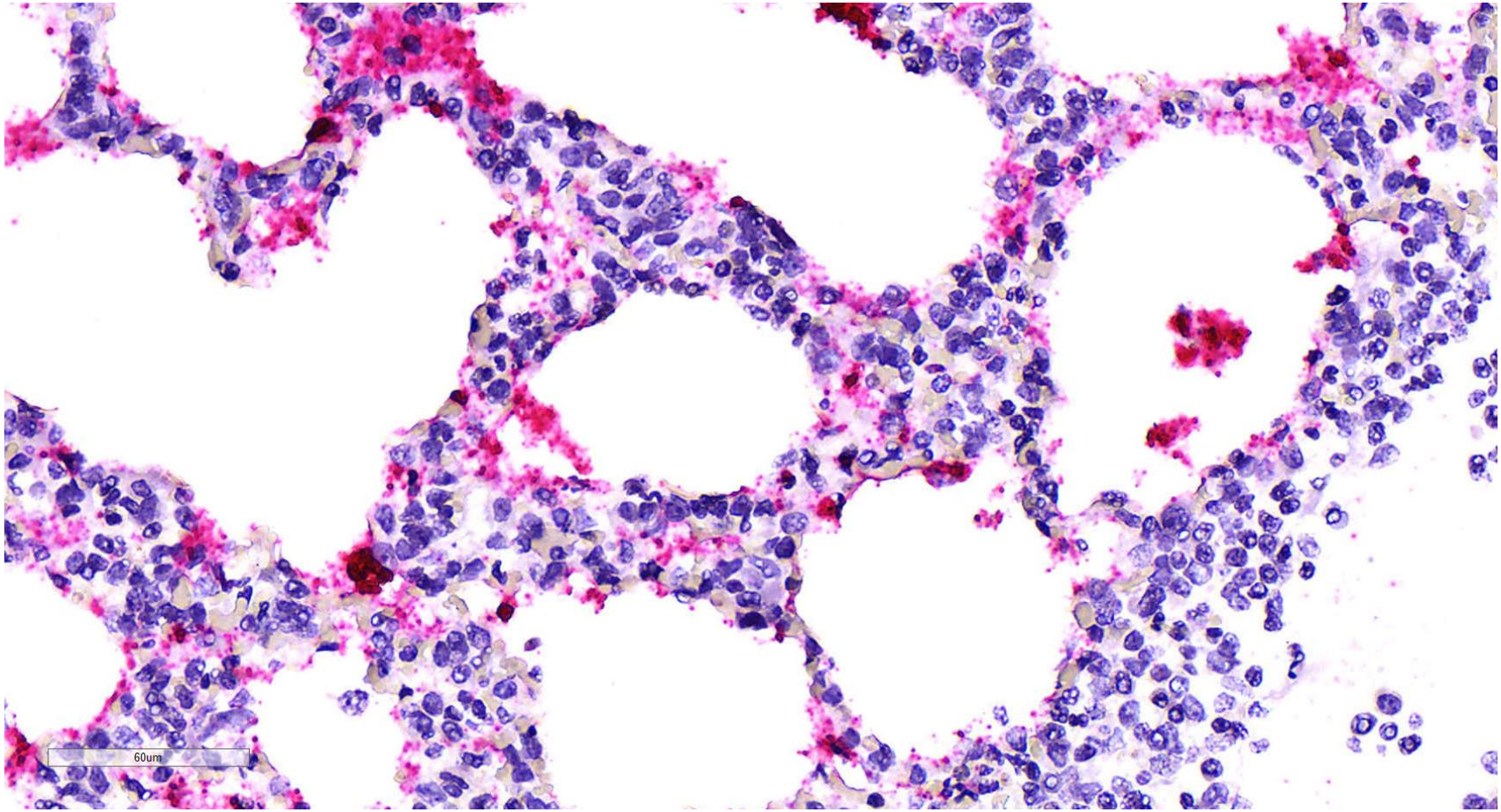

Figure 5 – Lung, Hybridization signals in the cytoplasm of cells present in the interalveolar septa demonstrating high levels of PRRSv replication within alveolar macrophages and type II pneumocytes.