RESEARCH ABSTRACT

SWINE HEALTH

**Title:** Identification of Type I Interferon Antagonists of PRRSV Viral Structural Proteins **– NPB #07-108**

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**Scientific Abstract:**

PRRSV has been known to suppress type I interferon production, but the exact mechanism is still unknown. Type I interferons (INFα and INFβ) play an important role in early innate antiviral immune responses and initiation adaptive immune responses. This project was intended to identify PRRSV structural protein(s) that may counteract the immune response by serving as type I interferon antagonist(s). To achieve this goal, PRRSV structural proteins were initially cloned in pcDNA plasmid vectors that can support production of encoded proteins in eukaryotic cells, but no protein expression was detected. As a result, other plasmid vectors were investigated. One plasmid, pCI, was eventually identified as suitable for cloning and expression of PRRSV proteins. In addition, a Flag-tag was engineered at the C-terminal end of each protein to facilitate identification in transfected eukaryotic cells. Individual structural and nonstructural proteins of PRRSV have now been successfully expressed in Marc-145 cells. We also investigated several variations of the proposed screening method to be utilized in identification PRRSV proteins that may act as type I interferon antagonists, downregulating the robustness of the innate immune response. We concluded that the proposed method was not suitable for our purposes. Instead, we are now collaborating with Dr. Laura Miller, who has established confirmatory assays for the activity of type I interferon (IFN-α and IFN-β) at the National Animal Disease Center. Specifically, these tests include a bioassay based on interferon stimulation of Mx1 gene transcripts, interferon-alpha and interferon-beta (IFN-α and IFN-β) gene transcriptional assays (real-time RT-PCR), and immunoassays (ELISA) for IFN-α and IFN-β. This screening system for type I interferon has now been established with recombinant attenuated Newcastle disease virus with an incorporated gene for the green fluorescent protein (rNDV/GFP), a positive indicator of type I interferon induction. We are now testing the PRRSV protein transfected Marc-145 cell supernatants for interference with rNDV-GFP type I interferon induction.