RESEARCH ABSTRACT

SWINE HEALTH

Title: Propagation of PEDv in tissue culture and development of standardized reference samples for use in diagnostic testing **- NPB #13-222**

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Scientific Abstract:
The highly contagious and deadly porcine epidemic diarrhea virus (PEDv) first appeared in the US in April 2013. Since then the virus has spread rapidly nationwide causing high mortality among nursing piglets and significant economic losses. Currently there are no efficacious preventive measures or therapeutic tools to control PEDv: therefore, development of an effective PEDv vaccine for swine in the US should be a high priority. Since most viruses accumulate mutations and become attenuated (reduced pathogenicity) after serial passage in cell culture, the isolation of PEDv in cell culture is the first step toward the development of an attenuated vaccine and to study the basic biology of PEDv and to develop *in vitro* PEDv immunoassays, inactivation assays and screen for PEDv antivirals. Our labs have started to work on PEDv since June, 2013. What we have achieved on PEDV research is listed below:

1. We have successfully passaged one Ohio PEDv strain PC22A in Vero cells. Currently PC22A strain replicates in Vero cells consistently and infectious virus titers reach 5-6 $\log_{10}$ 50% tissue culture infectious dose ($TCID_{50}$) or plaque forming units (PFU) per mL. **The isolation of PEDv in cell culture is the first step toward the development of an attenuated vaccine. Our tissue cultured (TC) PEDv has adequate infectious titers to be developed as an inactivated booster vaccine to enhance lactogenic immunity in sows previously infected with PEDv to control endemic PED. It also can be used to evaluate disinfection/decontamination efficiency *in vitro* to promote the control of virus spread.** Our long term goal is to continue to passage this strain to develop a live attenuated PEDv vaccine for use in naïve PEDv seronegative sows to protect their nursing piglets against epidemic PED.
2. We have plaque-purified PC22A at a low cell culture level (passage level 3) using plaque assays, confirmed that it retained high virulence similar to the wild-type PEDV PC21A strain, and generated a virus pool with high infectious titers (7.7 $\log_{10}$ PFU/mL) in germfree pigs. **It can be used as a challenge pool in future vaccine studies.**

3. We have developed conventional reverse-transcription (RT)-PCR and real-time RT-PCR (RT-qPCR) and immunofluorescent assays (IFA) for the detection of PEDv nucleic acids and antigens, respectively. TC PEDv-based immunofluorescent assay (IFA) and plaque reduction virus neutralization assay (VN) have been developed for the detection of isotype (IgA and IgG) and virus neutralizing antibodies, respectively, from pig serum samples. **Such assays are essential to: 1) screen fecal and serum samples from swine herds for PEDv prevalence; 2) detect antibodies to PEDv to determine pigs' PEDv infection status or to permit their export if PEDv sero-negative status is required; and 3) evaluate whether a vaccine induces protective immunity.**
4. We have investigated the pathogenesis of US PEDv in nursing germfree pigs. By using gnotobiotic (Gn) animals, the precise role of a single pathogen in the disease process can be evaluated. We found that the germfree pig-passaged Ohio PEDv PC21A strain were free of other bacteria and enteric viruses. The virus pool can be used as challenge pool in the future vaccine studies. The infected pigs exhibited acute severe diarrhea/vomiting between 24-48 hpi, followed by dehydration and collapse. Pathologic lesions were limited to the small and large intestines, although mainly in the jejunum and ileum. Severe atrophic enteritis was identified microscopically. All infected pigs had viral RNA detected in feces and serum. Overall, our data suggest that the **US PEDv PC21A strain acutely infects the entire intestine, leading to severe atrophic enteritis, and is highly enteropathogenic.**