RESEARCH ABSTRACT
SWINE HEALTH

Title: **Ex-vivo bioassay method to assess viral infectivity in feeds and non-traditional sample matrices - NPB #14-160**

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Scientific Abstract

Virus-contaminated feed ingredients has been suggested as a source of porcine epidemic diarrhea virus (PEDV) to US and Canadian swine and as a contributing factor to rapid regional and national spreading of the virus. While positive PCR finding of PEDV RNA in feed or feedstuffs raised the serious question about the cleanness of environment at both collection and manufacturing sites and manufacturing quality control processes of raw materials to supply and storage of final products, infectivity of such PCR-positive materials in pigs has been proven extremely difficult through feeding trials, which raised the need for a different and reliable way to assess the infectivity of these materials. PEDV has been known to be difficult to isolate or propagate in cell culture (less than 10% success rate). Alternatively, swine bioassay is an excellent tool to access infectivity as pigs are the natural hosts. However, swine bioassay is resource-driven (i.e., expensive and labor-intensive), is a biologically variable system which can be confounded by many factors, and takes longer turnaround.

Small intestine is the ultimate place for enteric viral pathogens to replicate, leading to the disease due to functional disruption of villi and other parts. We explored an ex-vivo bioassay for PEDV in feed or feedstuffs using "*Tied Small Intestinal Segment* (TSIS)" by maintaining the intact structure of intestine outside of the pig under laboratory conditions. A TSIS was a cross-sectional piece of small intestine with both ends tied-off and liquid material (i.e., inoculum) injected into the lumen, which was then kept moist by immersing in sterile cell culture media within a sterile petri dish and maintained at 37°C in a humidified CO_2 incubator to mimic the body condition. Hence TSIS could be considered to be a miniaturized natural pig gut with functional enterocytes along with villous structure. The specific objective of the study was to determine if TSIS from neonatal pigs can be a cost-effective diagnostic tool for rapid and reliable determination of infectivity. Specific aims were:

1. To assess how long TSIS can be maintained in-vitro without detrimental loss or alteration of villous structure;
2. To determine if TSIS permissive to coronaviruses and rotaviruses and all intestinal segments are equally susceptible to these viruses; and
3. To characterize how sensitive is TSIS method for viral infectivity assay.

Each TSIS could be inoculated with pp to 2ml of sample. TSIS could be maintained *in vitro* up to 72 hours without significant loss of enterocytes and villous structure under optimized conditions regardless of sample matrices (i.e., media or feed extracts) tested. Virus infection and growth in TSIS was evident to a degree by PCR or IHC when inoculated with cell-culture derived PEDV, TGEV or porcine rotavirus A containing as low as 10^2 PFU/ml at 72 hours post inoculation (PI) but not at 48 hours PI. Jejunum and ileum were permissive to the viruses tested regardless of source of intestine (i.e., CDCD or snatch-farrowed piglets). When feed or feed ingredients positive for PEDV RNA with Ct values of 28-34 were tested in TSIS, no infectivity was detected. Some of the samples which were tested by swine bioassays also did not show the presence of infectious PEDV.

In conclusion, this proof-of-concept study suggests that TSIS could be an ex-vivo bioassay tool to measure the presence of infectious viruses in feed or feedstuffs. While this new method appeared to be better than cell-culture based assessment, TSIS method was not sensitive enough under study conditions to detect the presence of PEDV at a low level which has been commonly the case with any of PEDV RNA positive feedstuffs tested at ISUVDL based on Ct values. Therefore, further optimization remains to enhance the sensitivity of TSIS-based testing for infectivity.