Title: Tissue localization, shedding, virus carriage, antibody response, and aerosol transmission of porcine epidemic diarrhea virus (PEDV) following inoculation of 4 week old feeder pigs

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Scientific Abstract:

The purpose of this investigation was to determine tissue localization, shedding pattern, virus carriage, antibody response, and aerosol transmission of PEDV following oral/nasal inoculation of 4-week-old feeder pigs. Thirty-three PEDV naive 3-week-old feeder pigs obtained from a high health commercial source were allowed to acclimate for one week prior to inoculation. The study was conducted under BSL3Ag containment at the Biosecurity Research Institute at Kansas State University. Twenty-three 4-week-old pigs (Group A) were inoculated with a “feedback” inocula used for controlled exposure of a sow herd via the oral and intranasal routes with 5 ml of inocula per route. Five pigs were not inoculated, but were comingled (Group B) with inoculated animals approximately 6 hours post inoculation. Five aerosol transmission pigs (Group C) were not inoculated, but were housed in a separate pen in the common animal room. Nasal and fecal swabs, serum, and oral fluid samples were collected prior to challenge and subsequently at days 0-7, 9, 14, 21, 28, 35, and 42 days post inoculation (DPI). PEDV shedding was monitored by real-time PCR of fecal and nasal swab samples and oral fluids. Serum samples were collected in order to monitor viremia and antibody response. Fresh and formalin-fixed tissues were collected from randomly selected Group A pigs at days 0, 2, 4, 7, 9, 14, 21, 28, 35, and 42 DPI in order to monitor tissue tropism of the virus and histopathology. Following inoculation, the animals were also observed daily for clinical signs of disease. Experimental data indicate the following: mild clinical signs appeared on DPI 2 and resolved by DPI 8 in Group A and B pigs. Fecal and nasal swabs were PCR positive in the inoculated group by DPI 2. Peak fecal shedding occurred on DPI 5 and was significantly higher than nasal swabs. Most group A and B animals were PCR negative by fecal or nasal swab testing at 21 DPI; however, some animals shed virus as long as 35 DPI.

Atrophic enteritis was observed in the jejunum and ileum of affected piglets from 2 to 8 DPI, and corresponded to positive antigen detection by IHC. Mesenteric lymph node and small intestine were the primary sites of antigen detection by IHC and tissue qRT-PCR, and most inoculated group A piglets were qRT-PCR positive in the intestinal tissue samples out to the end of the study.
Tissue blocks were sent to Dr. Madson at ISU for PEDV preliminary immunohistochemistry (IHC) evaluation. IHC results were subsequently confirmed and results expanded at KSVDL. The only samples that tested positive for the presence of viral antigen were tissues from the GI tract. Turbinates, trachea, lung, bronchial lymph nodes, spleen, and other visceral tissues were all negative for PEDV as evaluated by IHC.

A complete set of serum samples has been provided to 5 laboratories (~1,200 samples) for assay development/standardization. In addition, 3 complete sets of oral fluid samples and tissues have been provided to other laboratories. These samples have been used for assay development and standardization across the different diagnostic laboratories.

Productive transmission did not appear to occur in the aerosol control group in spite of the PEDV nucleic acid detected in the nares of some of those animals and in the oral fluids. Room environmental samples collected at DPI 14 demonstrated that viral nucleic acid was abundant on the walls, pens, and food bins in the challenge room. PEDV viremia was clearly detected in 3 of the 5 contact controls and 9 of the 22 inoculated animals. No detectable viremia was detected in any of the aerosol control animals. Serological data (IFA) proves that pre-inoculation samples were negative and that there was significant seroconversion in all of the inoculated and contact control animals. There was no evidence of seroconversion in the aerosol control group. These results seem to be in conflict with reports from the field that implicate aerosol transmission, but lack confirmation via bioassay. Factors like disinfectant and ultraviolet inactivation of PEDV, sensitivity of the indicator animal (nursing pigs vs. weaned pigs) and infectious dose as a function of route of exposure need to be investigated in order to gain insight into modes of transmission of PEDV.

The tissue PCR positivity for PED nucleic acid at day 43 post inoculation was an unexpected finding which provides insight into virus carriage and potential transmission of the virus long after the clinical disease has abated. In view of these findings, additional animal co-mingle studies will need to be conducted to determine the actual duration of horizontal transmission between infected and naïve pigs.