Progress Report of
NPB Sponsored PEDV Research—Pig Inoculation

Project Objectives.
Determine tissue localization, shedding pattern, virus carriage, antibody response, and aerosol transmission of Porcine Epidemic Diarrhea Virus (PEDV) following inoculation of 4-week-old feeder pigs.

Multiple aliquots of all samples collected will be shared with requesting laboratories in order to expand diagnostic testing and vaccine development capabilities.

Methods and Materials.
Experimental Animals: Thirty-three PEDV naive 3-week-old feeder pigs obtained from a high health commercial source were used in this investigation. The animals were allowed to acclimate for one week prior to inoculation. The study was conducted under BSL2 containment at the Biosecurity Research Institute at Kansas State University.

Numbers/Grouping: Group-A pigs were inoculated with the PEDV challenge material. Group B pigs were not inoculated, but were co-mingled with inoculated Group A animals approximately 6 hours after inoculation. The aerosol transmission Group-C pigs were not inoculated, but were housed in a separate pen in the common animal room as Groups A and B.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th># of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PEDV oronasal inoculated</td>
<td>23</td>
</tr>
<tr>
<td>B</td>
<td>None—Contact Control</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>None—aerosol transmission controls</td>
<td>5</td>
</tr>
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Challenge: The challenge material was a pool of gut derived intestinal contents that has been used as "feedback" inocula for controlled exposure of a sow herd in a commercial swine production unit. The challenge material was kindly provided by Dr. Matt Ackerman of Swine Veterinary Services. The inocula had a PEDV nucleic acid "CT titer" of 22 in the KSVDL real-time PCR assay. Challenged animals (Group A) were inoculated at 4 weeks of age via intranasal and oral routes with 5 ml of inocula per route.
**Sampling Requirements/Challenge Scheduling:** The animals were observed daily for clinical symptoms. Nasal and fecal swabs and serum samples were collected prior to challenge and days 0-7, 9, 14, 21, 28, 35 and 42 post challenge. Pen oral fluid samples were also collected at the same time points for Groups A/B and the aerosol control Group C.

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.

Following euthanasia, fresh and formalized tissues were collected from randomly selected Group A pigs at days 0, 2,4,7,9,14,21,28, 35 and 42 post challenge. The samples were collected in order to monitor tissue tropism of the virus and histopathology.

**Preliminary Results as of 30 August 13:**

Preliminary fecal and nasal shedding PCR results are presented in the graph below. The graph represents Group average cycle thresholds (CTs) with an inverse relationship between the CT value and the amount of virus present. A CT value of 40 is considered negative.

![Fecal and Nasal Shedding following PEDV Inoculation of 4-Week old Pigs](image-url)
**Oral Fluid Shedding following PEDV Inoculation of 4-Week old Pigs**

![Graph showing oral fluid shedding following PEDV inoculation of 4-week old pigs.](image)

**Viremia Following PEDV inoculation of 4 Week Old Pigs**

![Graph showing viremia following PEDV inoculation of 4-week old pigs.](image)
The data indicates the following:

1. Surprisingly, all samples were negative for the virus at 24 hours post inoculation.
2. Fecal and nasal shedding of the inoculated group (A) was first observed at 48 hours post inoculation.
3. Nasal shedding was detected in the Contact Control group (B) at 48 hours post inoculation and fecal shedding occurred 24 hours later.
4. Peak fecal shedding occurred 5 to 6 days post challenge and was significantly higher than nasal shedding.
5. Oral Fluids from Inoculated animals (Group A) were PCR positive at 48 hours post inoculation.
6. Serum samples demonstrated the presence of Viremia following intranasal/oral inoculation.
7. Most inoculated (A) and contact control (B) animals were not shedding intranasal virus at 21 days post inoculation.
8. In Groups A and B, the majority of the animals were negative for fecal shedding at 21 days post inoculation. However, 3 of 11 animals in the inoculated group and 1 of 5 animals in the contact control group were still shedding virus at 21 days post inoculation and 1 of 11 was positive at 28 days post inoculation.
9. Productive aerosol transmission (Group C) did not appear to occur in spite of the fact that PEDV nucleic acid could be detected in the nares of some of the animals at 5 and 7 days and oral fluids post inoculation of the Group A animals.

Additional testing is currently underway for viremia, oral fluid shedding, in situ tissue localization and antibody response. Results will be shared as they become available.