

## **PEDV Research Updates 2013**

Porcine Epidemic Diarrhea virus (PEDV) has caused significant challenges to the swine industry. The virus had not been previously identified in the United States prior to April of 2013. To assist producers and their veterinarians in the management, control and potential elimination of the virus, the National Pork Board funded key research projects to better understand PEDV. In order to provide timely information to producers from those projects, the objectives and initial updates will be periodically reported.

**NOTE:** The updates from the proposal represent interim information only and are not intended to be a final report. The final and formal reports will be provided at the end of the terms of the projects and then posted online at [pork.org](http://pork.org). The update information is intended to inform stakeholders of progress but are not intended to be the final outcome. For further information, please contact Dr. Lisa Becton at [lbecton@pork.org](mailto:lbecton@pork.org).

### **#13-228: Kansas State University**

**Tissue localization, shedding, virus carriage, antibody response, and aerosol transmission of Porcine Epidemic Diarrhea Virus (PEDV) following inoculation of feeder pigs.**

#### **Objectives:**

Study of the basic pathogenesis and characterization of the virus: Tissue localization, shedding, virus carriage, antibody response, and aerosol transmission of Porcine Epidemic Diarrhea Virus (PEDV) following inoculation of feeder pigs will be investigated. In an attempt to expand diagnostic testing capabilities, multiple aliquots of all will be samples collected and shared with requesting laboratories

#### **Update: 11-25-13**

No new information is available this week. Project is ongoing.

#### **Update: 11-13-13**

In Situ hybridization (ISH) development is still underway at KSVDL. The ISH development effort is very preliminary and no results are available at this time. When completed, the ISH and IHC methods will be compared for sensitivity and possible differences of PEDV tissue tropism.

Large quantities of characterized PED virus stock have been prepared and titrated for the serum neutralization assay development. A rabbit anti PEDv "M" antisera has been provided by Dr. Ying Fang and will be used as the PEDv indicator to monitor neutralization of input virus in the SN test. Direct comparisons with the indirect fluorescent antibody test will be made once the SN test is standardized.

A complete set of weekly samples from the study (fecal and nasal swabs, oral fluid samples and serum samples) is being sent to NVSL for assay comparison/development/validation.

Virus isolation attempts on frozen intestinal tissue samples that have previously been provided to three laboratories is still underway—sequential passage attempts have been unsuccessful thus far.

#### **Update: 10-28-13**

We have finished the first round of PEDV detection in tissues. Tissue blocks were sent to Dr. Madson at ISU for PEDV immunohistochemistry (IHC) evaluation. The only samples that tested positive for the presence of viral antigen were tissues from the GI tract. In Situ hybridation (ISH) development is currently underway at KSVDL. The ISH development effort is just getting started and no results are available at this time. When completed, the ISH and IHC methods will be compared for sensitivity and possible differences of PEDV tissue tropism.

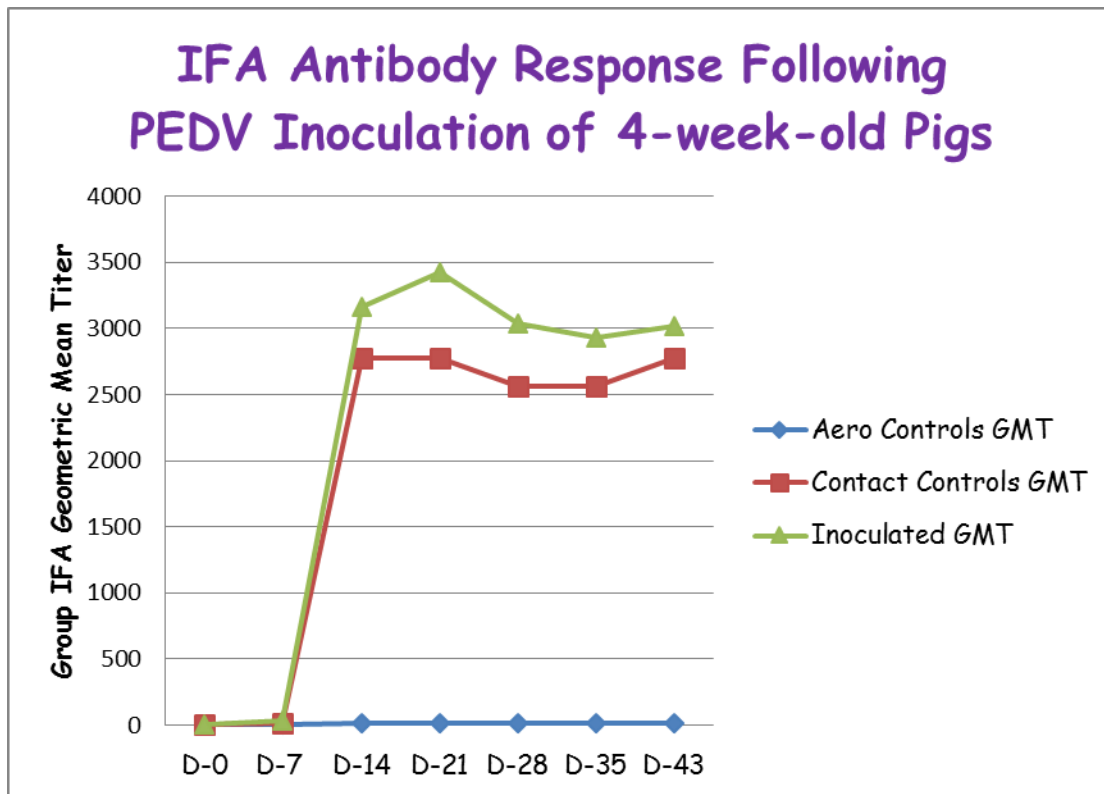
Large quantities of characterized PED virus stock are being prepared for serum neutralization assay development and indirect fluorescent antibody test standardization.

Frozen intestinal tissue samples have been provided to three laboratories for virus isolation optimization. One additional set of oral fluids has also been provided to a requesting lab.

**Update: 10-16-13**

We have finished the round first IFA testing of the complete set of serum samples from the inoculation experiment. Antibody titers were higher than expected in some of the samples so the mid-point of the next higher dilution was used as the value to calculate geometric mean titers for the graph below. Actual titers for all samples will be determined and the graph will be corrected as the data becomes available.

Thus far, a complete set of serum samples have been provided to 4 laboratories (~800 samples) for assay development/standardization. In addition, a complete set of oral fluid samples have been provided to a requesting lab.



**Update: 10-3-13**

### 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.

Group	Treatment	# of Animals
A	PEDV oronasal inoculated	23
B	None—Contact Control	5
C	None—aerosol transmission controls	5

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.

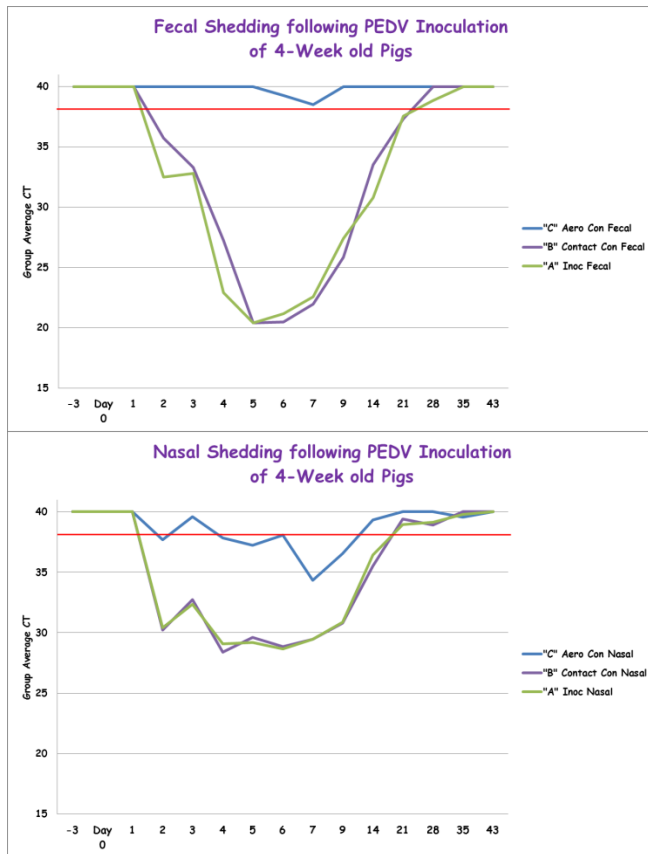
Sampling Requirements/Challenge Scheduling: The animals were observed daily for clinical symptoms. Nasal and fecal swabs and serum samples were collected prior to challenge and days 0-7, 9, 14, 21, 28, 35 and 42 post challenge. Pen oral fluid samples were also collected at the same time points for Groups 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.

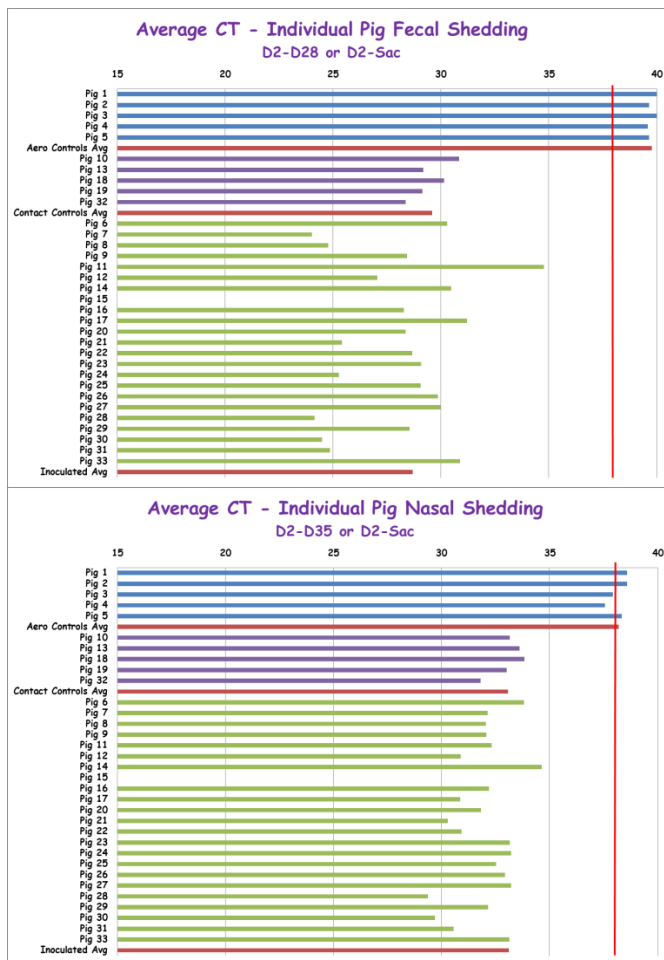
Following euthanasia, fresh and formalized tissues were collected from randomly selected Group A pigs at days 0, 2,4,7,9,14,21,28, 35 and 42 post challenge. The samples were collected in order to monitor tissue tropism of the virus and histopathology.

**Preliminary Results as of 3 Sept 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.



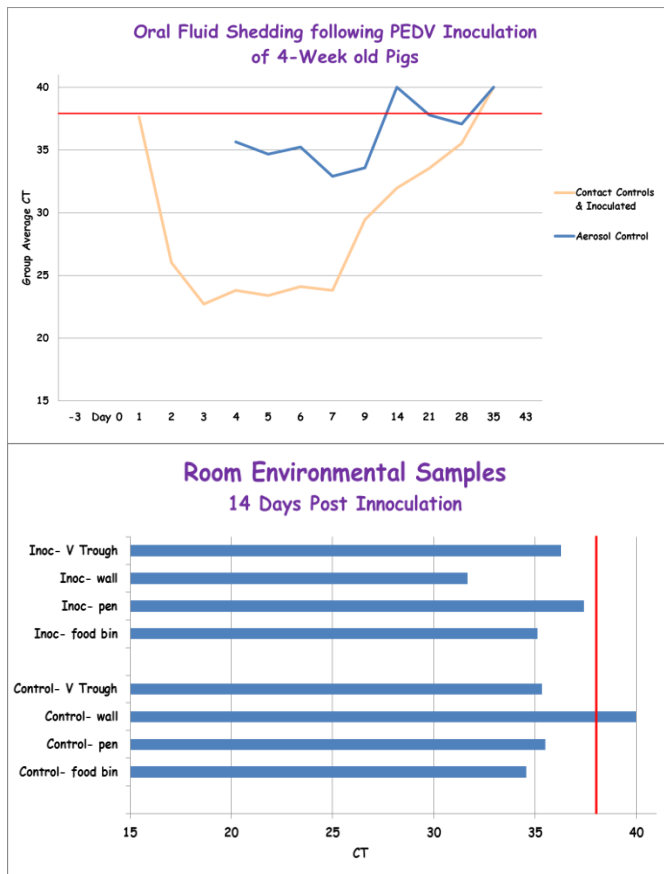
- Surprisingly, all samples were negative for the virus at 24 hours post inoculation.
- Fecal and nasal shedding of the inoculated group (A) was first observed at 48 hours post inoculation.
- Nasal shedding was detected in the Contact Control group (B) at 48 hours post inoculation and fecal shedding occurred 24 hours later.
- Peak fecal shedding occurred 5 to 6 days post challenge and was significantly higher than nasal shedding.
- 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.
- Most inoculated (A) and contact control (B) animals were not shedding intranasal virus at 21 days post inoculation.



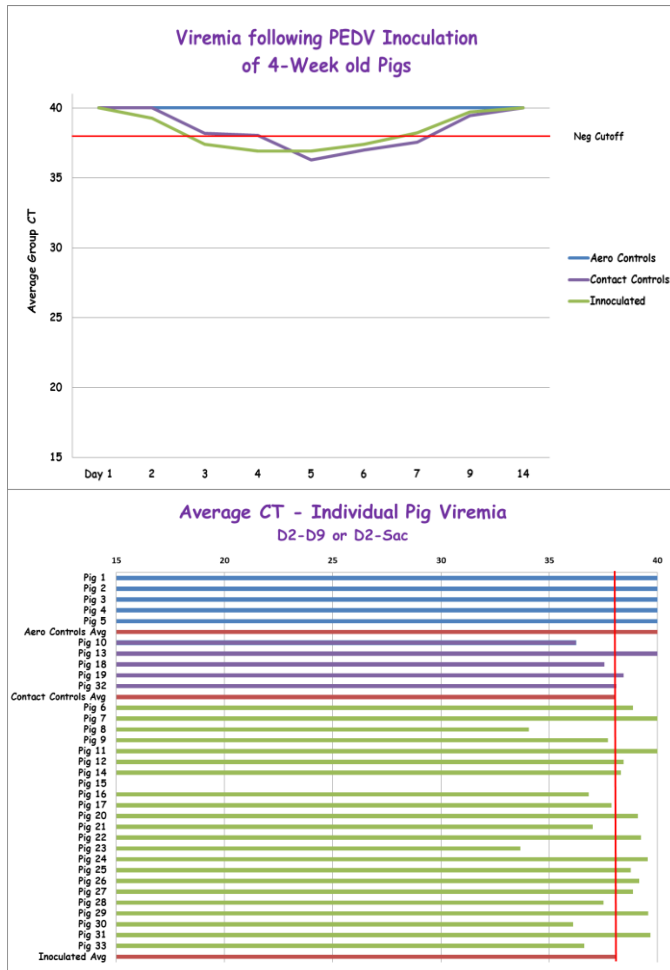
The graphs above are designed to look at individual shedding characteristics of pigs in the three different experimental groups. The data is presented as the average CT value over a defined shedding period; the lower the CT, the greater amount of virus that is shed over that time frame.

Due to the small number of experimental animals in the various groups, caution should be applied when making interpretations.

- There appears to be considerable variation in fecal shedding patterns among inoculated pigs.
- Fecal shedding patterns among contact controls appear to be more consistent.
- Fecal shedding appeared to be absent in each of the aerosol control animals.
- Nasal shedding patterns were less variable than fecal shedding patterns.
- Based on a CT cut off value of 38, two of the five aerosol control animals appeared to have viral nucleic acid in their nares. These same animals showed no evidence of fecal shedding.



- Oral Fluids from the pen housing Inoculated animals (Group A) and contact controls (Group B) were PCR positive at 48 hours post inoculation and remained positive until day 28 post inoculation.
- Oral fluids from the aerosol control group appeared to be positive at the time of the first successful collection point (D-4) and they remained positive through day 28 post inoculation.
- A weak CT (>37) was observed in the aerosol group at days 21 and 28 post inoculation.
- Room environmental samples were collected at 14 days post inoculation-the data demonstrate that viral nucleic acid was abundant on the walls, pens and food bins on both the inoculated and aerosol control areas in the challenge room.
- Due to the possibility of a false positive PCR reaction, questionable samples were retested and the reaction products were sequenced to determine if the product was PEDV specific. All questionable reactions demonstrated the presence of PEDV viral nucleic acid.



- 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.
- The raw data suggest that there seems to be a correlation with viremia and extended duration of shedding either fecal or nasal.

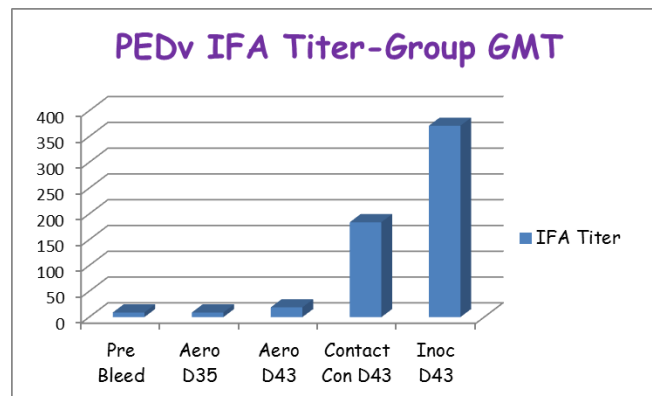
Fecal, nasal and oral fluid viral nucleic acid detection data via real time PCR clearly demonstrate productive PEDV infection in the Inoculated and Contact Control groups in this study. Pigs that were positive for nasal shedding were also positive for fecal shedding-most of the time at a 10 fold or greater level than that observed in the nares. Viral nucleic acid levels in oral fluids were in between those observed in both fecal and nasal samples.

In contrast, fecal shedding was not demonstrated in the aerosol control group but samples did test positive for the presence of PEDV viral nucleic acid in nasal and oral fluid samples.



Serological testing of selected serum samples has recently been completed. The graph below depicts the geometric mean indirect fluorescent antibody test results from the day 43 sera of all experimental groups, day 35 sera from the aerosol control group and selected pre-inoculation sera from infected animals.

- The data show pre-inoculation samples are negative and that there was significant seroconversion in the all of the inoculated and contact control animals.
- There is no evidence of seroconversion in the aerosol control group at day 35 or 43 in spite of the clear demonstration of PEDv nucleic acid in nasal and oral fluid samples.
- The IFA data was in complete agreement with an E. coli expressed NP ELISA (96 well format) that is being developed.
- Additional serological assays currently under development and optimization include a multiplex Luminex assay and a serum neutralization assay.



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

The experimental results demonstrate that aerosol transmission did not occur in this study. 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.

#### **Update: 9-21-13**

We are busy analyzing our data and correlating it with serology response. The serology is coming along nicely, but we are still optimizing. We are preparing 5 complete sets of weekly serum aliquots for all of the animals for the entire study (roughly 1200 aliquots) that will be shared with other laboratories for assay development and standardization. We are also preparing large quantities of positive and negative

control sera that can serve as reference standards. Next update will be available the week of October 1<sup>st</sup>.

**Update: 9-2-13**

**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.

The following new results are from pigs that are 4 weeks of age.

- 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.
- 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.

**Quick Take**

- The objective is to determine tissue localization, shedding pattern, virus carriage, antibody response, and aerosol transmission of PEDV following inoculation of 4-week-old feeder pigs.

**Update: 8-21-13**

**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.

<b>Group</b>	<b>Treatment</b>	<b># of Animals</b>
A	PEDV oronasal inoculated	23
B	None—Contact Control	5
C	None—aerosol transmission controls	5

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.

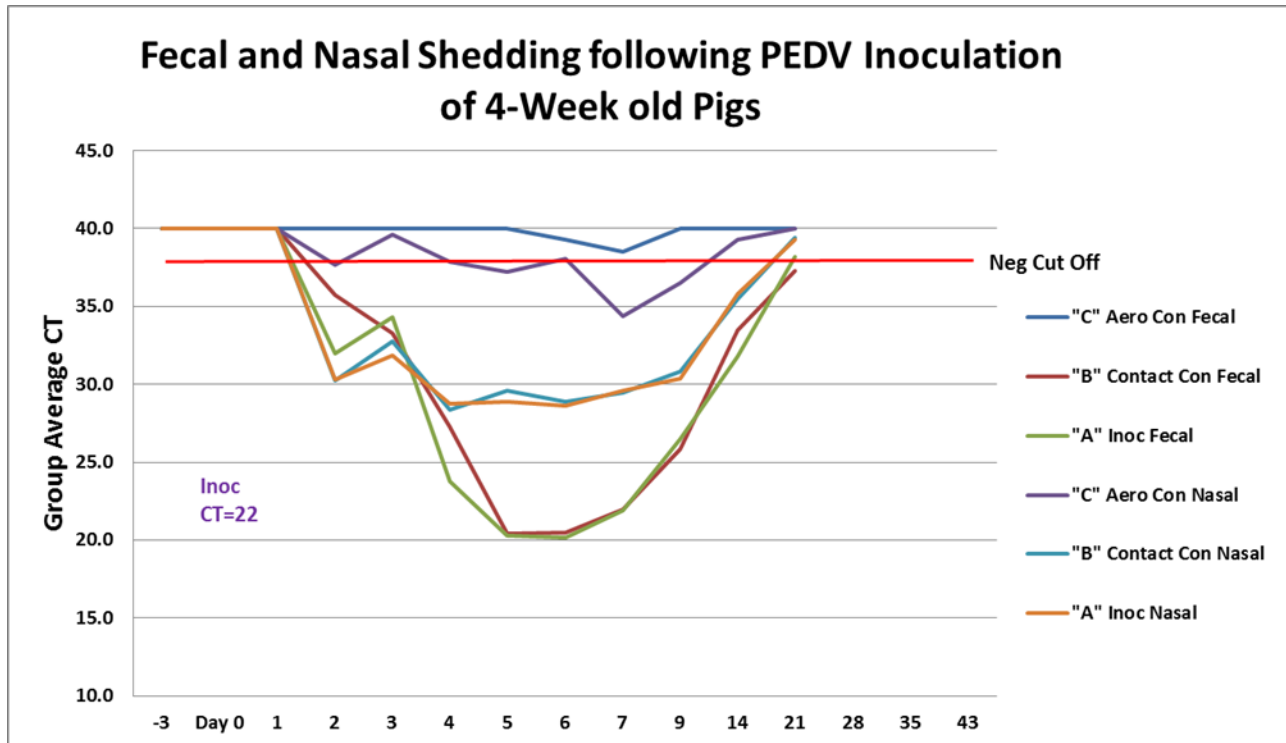
Sampling Requirements/Challenge Scheduling: The animals were observed daily for clinical symptoms. Nasal and fecal swabs and serum samples were collected prior to challenge and days 0-7, 9, 14, 21, 28, 35 and 42 post challenge. Pen oral fluid samples were also collected at the same time points for Groups 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 14 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.



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. Most inoculated (A) and contact control (B) animals were not shedding intranasal virus at 21 days post inoculation.
6. 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 that time point.
7. 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 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.