

## 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).

### #13-215: University of Minnesota Environmental stability of PED (porcine epidemic diarrhea virus)

#### Objectives:

Determine the environmental stability of PED (porcine epidemic diarrhea virus):

Aim 1. To determine survival of PED in fresh feces that represents the risk posed by transport.

Aim 2: To determine survival of PED in slurry (old feces in the pit) that reflects the risk of manure spreading.

Aim 3. To study PED survival in drinking and recycled water (truck washes).

Aim 4. To study PED survival in animal feed.

#### Update: 1-21-14

#### Environmental stability of PEDV; PI: Sagar Goyal

- 1. Survival of PEDV in fresh feces:** Samples of fresh porcine feces were spiked with PEDV and then stored at 40°C, 50°C and 60°C under three different levels of relative humidity (RH): 30%, 50%, and 70%. The results show that PEDV can survive for up to 7 days at these temperatures. The survival of TGEV (transmissible gastroenteritis virus, a related coronavirus) was determined under similar conditions and its survival was similar to that of PEDV.
- 2. Survival of PEDV in slurry:** Samples of slurry were spiked with PEDV and then stored at room temperature (~25°C), 4°C, and -20°C. The samples at ~25°C and 4°C were kept at three different levels of relative humidity (RH): 30%, 50%, and 70%. The PEDV survived for 28 days (last time the samples were tested) sampling at 4°C and -20°C at all three levels of RH. At room temperature, PEDV survived for 14 days. Similar results were obtained with TGEV.
- 3. Survival of PEDV in drinking and recycled water:** Samples of drinking and recycled water from PEDV negative farm were autoclaved and spiked with PEDV and then stored at various times at room temperature ~25°C (5 mL aliquot of water spiked with 500 µl of virus in triplicate). Infectious PEDV was detected after 1 week in both drinking and recycled water.
- 4. Survival of PEDV in animal feed:** Wet feed was prepared by adding 10 mL of PBS to 5 gm of dry feed. The wet slurry was spiked with PEDV, stored at room temperature, and tested at various time intervals. At room temperature, PEDV RNA was not degraded in wet feed for 28 days. In dry feed, however, the virus survived for only 1 week but not for 2, 3, 4, and 5 weeks. The results obtained with TGEV were also similar.
- 5. Infectious dose of PEDV:** To determine the infectious dose of PEDV, we inoculated serial 10-fold dilutions of PEDV (clarified homogenate of intestinal mucosa from a PEDV-infected piglet) in 10-day-old piglets. Clinical signs of gastroenteritis were seen in piglets inoculated with 10<sup>-2</sup> to 10<sup>-8</sup> dilutions. No clinical signs were seen in piglets inoculated with 10<sup>-9</sup> to 10<sup>-12</sup> dilutions. These results indicate that the infectious dose of PEDV is very low.

Update: 12-12-13

**Project 2: Environmental stability of PEDV**

**PI: Sagar Goyal**

**Survival of PEDV in fresh feces:** Samples of fresh feces were spiked with PEDV and then stored at 40°C, 50°C and 60°C under three different relative humidity (RH) levels; 30%, 50%, and 70%. The results of bioassay on these samples are shown in Tables 1, 2 and 3.

**Table1:** Survival of PEDV in fresh feces at 40°C

| RH levels | Time (days) | Initial cT value | Extent of diarrhea in piglets | cT value in intestinal samples from inoculated piglets |
|-----------|-------------|------------------|-------------------------------|--|
| 30%       | 0           | 24.13            | 2+                            | 16.48  |
|           | 3           | 27.29            | 1+                            | 32.00  |
|           | 7           | 30.54            | 0                             | -  |
|           | 14          | 43.07            | 0                             | -  |
|           |             |                  |                               |  |
| 50%       | 1           | 27.59            | 2+                            | 16.35  |
|           | 3           | 28.70            | 1+                            | -  |
|           | 7           | 31.09            | 0                             | -  |
|           | 14          | 36.21            | 0                             | -  |
|           |             |                  |                               |  |
| 70%       | 0           | 27.36            | 2+                            | 13.79  |
|           | 3           | 32.74            | 1+                            | 13.30  |
|           | 7           | 37.92            | 1+                            | 15.16  |
|           | 14          | -                | 0                             | -  |
|           |             |                  |                               |  |

**Table 2:** Survival of PEDv in fresh feces at 50°C

| RH levels | Time (days) | Initial cT value | Extent of diarrhea in piglets | cT value in intestinal samples from inoculated piglets |
|-----------|-------------|------------------|-------------------------------|--|
| 30%       | 0           | 22.68            | 2+                            | 15.65  |
|           | 1           | 26.99            | 2+                            | 15.75  |
|           | 3           | -                | 0                             | -  |
|           | 7           | -                | 0                             | -  |
|           |             |                  |                               |  |
| 50%       | 0           | 21.85            | 2+                            | 16.63  |
|           | 3           | 31.63            | 2+                            | 19.08  |
|           | 7           | 37.85            | 1+                            | -  |
|           | 14          | -                | 0                             | -  |
|           |             |                  |                               |  |
| 70%       | 0           | 20.24            | 2+                            | 16.12  |
|           | 3           | 32.30            | 0                             | 14.99  |
|           | 7           | 33.79            | 0                             | 35.33  |
|           | 14          | -                | 0                             | -  |
|           |             |                  |                               |  |

**Table 3:** Survival of PEDv in fresh feces at 60°C

| RH levels | Time (days) | Initial cT value | Extent of diarrhea in piglets | cT value in intestinal samples from inoculated piglets |
|-----------|-------------|------------------|-------------------------------|--|
|-----------|-------------|------------------|-------------------------------|--|

|     |   |       |    |       |
|-----|---|-------|----|-------|
| 30% | 1 | 23.58 | 2+ | 13.26 |
|     | 3 | 29.08 | 0  | 35.10 |
|     | 7 | -     | 0  | 37.24 |
|     |   |       |    |       |
| 50% | 0 | 22.16 | 1+ | 17.94 |
|     | 1 | 31.03 | 2+ | 16.31 |
|     | 3 | 32.00 | 0  | -     |
|     | 7 | -     | 0  | -     |
|     |   |       |    |       |
| 70% | 1 | 33.34 | 0  | 33.93 |
|     | 3 | 34.50 | 0  | 35.61 |
|     | 7 | -     | 0  | -     |

**Update: 11-27-13**

**Project 2: Environmental stability of PEDV**

**PI: Sagar Goyal**

**Survival of PEDV in slurry:** Samples of slurry spiked with PEDV were stored at 25°C and 4°C under three different relative humidity (RH) levels; 30%, 50%, and 70%. At -20°C, PEDV RNA was detectable for 28 days and so was TGEV RNA as mentioned in the previous report. The results of bioassay on these samples are shown in Tables 1 and 2.

Table 1: Survival of PEDV in slurry stored at room temperature (~25°C) as determined by bioassay in 10-day-old piglets.

| RH level | Time (days) | Initial cT value | Extent of diarrhea in piglets | cT value in intestinal samples from inoculated piglets |
|----------|-------------|------------------|-------------------------------|--|
| 30%      | 0           | 20.30            | 2+                            | 17.01  |
|          | 7           | 21.17            | 1+                            | 15.81  |
|          | 14          | 22.17            | 1+                            | 36.86  |
|          | 28          | 23.54            | 0                             | -  |
| 50%      | 7           | 21.83            | 1+                            | 17.60  |
|          | 14          | 23.51            | 1+                            | 17.56  |
|          | 28          | 24.08            | 0                             | -  |
| 70%      | 7           | 21.01            | 2+                            | 15.75  |
|          | 14          | 21.41            | 1+                            | 35.86  |
|          | 28          | 21.51            | 0                             | -  |

These results indicate that infectious virus survives for at least 14 days in slurry stored at 25°C. These results also indicate that there is no effect of RH on virus survival.

Table 2: Survival of PEDV in slurry stored at 4°C as determined by bioassay in 10-day-old piglets.

| RH level | Time (days) | Initial cT value | Extent of diarrhea in piglets | cT value in intestinal samples from inoculated piglets |
|----------|-------------|------------------|-------------------------------|--|
| 30%      | 0           | 21.96            | 2+                            | 16.26  |
|          | 7           | 20.50            | 1+                            | 16.41  |
|          | 14          | 21.08            | 1+                            | 17.19  |
|          | 28          | 22.13            | 1+                            | 16.08  |
| 50%      | 7           | 20.77            | 2+                            | 17.03  |
|          | 14          | 21.29            | 2+                            | 16.04  |
|          | 28          | 21.95            | 1+                            | 17.90  |

|     |    |       |    |       |
|-----|----|-------|----|-------|
| 70% | 7  | 21.60 | 1+ | 16.19 |
|     | 14 | 22.26 | 2+ | 17.08 |
|     | 28 | 22.04 | 0  | 36.69 |

These results indicate that infectious virus survives for >28 days in slurry stored at 4<sup>o</sup>C at all three RH levels. **Note:** The results on survival of TGEV (for comparison) were similar to those obtained with PEDV.

**Update: 11-13-13**

**Project 2: Environmental stability of PEDV**

**PI: Sagar Goyal**

**Survival of PEDV in slurry:** At -20<sup>o</sup>C, PEDV RNA was detectable for 28 days and so was TGEV RNA. The results of bioassay, when slurry stored for various time periods was inoculated in piglets, are shown in Table 1.

Table 1: Survival of PEDV in slurry as determined by bioassay in 10-day-old piglets.

| Slurry stored at -20 <sup>o</sup> C for: | Initial cT value | Extent of diarrhea in piglets | cT value in intestinal samples from inoculated piglets |
|--|------------------|-------------------------------|--|
| 0 day                                    | 20.29            | 2+                            | 16.77  |
| 3 days                                   | 26.61            | 1+                            | 15.79  |
| 7 days                                   | 26.95            | 1+                            | 16.27  |
| 14 days                                  | 27.55            | 0                             | 15.51  |
| 28 days                                  | 28.56            | 0                             | 14.81  |

These results indicate that infectious virus survives for >28 days in slurry stored at -20<sup>o</sup>C

**Survival of PEDV in animal feed:** Two different experiments were done. In experiment 1, a slurry of feed was prepared in PBS followed by spiking it with PEDV. The samples were then removed at different time points to measure virus survival, if any. In the second experiment, the feed was used as such (dry feed, not slurry). Both of these experiments were done at room temperature (~24<sup>o</sup>C). When feed was used as slurry, PEDV RNA was not degraded for 28 days. The results of bioassay are shown in Table 2.

Table 2: Survival of PEDV in feed slurry by bioassay in 10-day-old piglets.

| Time of storage | Initial cT value | Extent of diarrhea | cT value in intestinal samples from inoculated piglets |
|-----------------|------------------|--------------------|--|
| 0 day           | 19.13            | 1+                 | 15.52  |
| 7 days          | 21.95            | 0                  | 15.21  |
| 14 days         | 23.36            | 0                  | 27.63  |
| 28 days         | 24.24            | 0                  | 29.67  |

**Note on Table 2:** Although no diarrhea was seen in piglets inoculated with feed stored for 1, 2, and 3 weeks, the positive PCR results on their intestinal mucosa suggest that the virus was still infectious after 28 days of storage.

Table 3: Survival of PEDV in dry feed as determined by bioassay in 10-day-old piglets

| Time of storage | Initial cT value | Extent of diarrhea | cT value in intestinal samples from inoculated piglets |
|-----------------|------------------|--------------------|--|
| 1 week          | 30.39            | 2+                 | 16.52  |
| 2 weeks         | 33.41            | 0                  | -  |
| 3 weeks         | 33.63            | 0                  | -  |
| 4 weeks         | 30.45            | 0                  | -  |
| 5 weeks         | 27.62            | 0                  | -  |

**Note on Table 3:** Diarrhea was seen in piglet inoculated with contaminated feed stored for 1 week but not with feed stored for 2, 3, 4, and 5 weeks indicating that the virus survives in dry feed for 1 week but not after 2 weeks.

**Note:** The results on survival of TGEV (for comparison) were similar to those obtained with PEDV.

**Update: 10-28-13**

Project 2: Environmental stability of PEDv

PI: Sagar Goyal

**Infectious dose of PEDv:** To determine the infectious of PEDv, we conducted two experiments. Serial 10-fold dilutions of PEDv (clarified homogenate of intestinal mucosa from infected piglet) were prepared in PBS and were tested by RT-PCR. The cT values are given in column 2 of Table 1. Column 3 shows the severity of diarrhea caused by different virus dilutions (from  $10^{-2}$  to  $10^{-7}$ ). Two days after virus inoculation, the pigs were killed, their intestinal mucosa collected, and tested for PEDv by PCR. cT values of this RT-PCR (in mucosa of inoculated piglets) are shown in column 4. The results indicate that PEDv is highly infectious and that the  $MID_{50}$  is very low.

| Virus dilution | Initial cT value | Extent of diarrhea | cT value in mucosal samples from inoculated piglets |
|----------------|------------------|--------------------|---|
| Undiluted      | 16.39            | ND*                | ND  |
| $10^{-1}$      | 17.73            | ND                 | ND  |
| $10^{-2}$      | 20.53            | ++                 | 17.24   |
| $10^{-3}$      | 23.55            | ++                 | 16.92   |
| $10^{-4}$      | 27.04            | ++                 | 15.32   |
| $10^{-5}$      | 29.94            | +                  | 17.10   |
| $10^{-6}$      | 32.06            | +                  | 16.02   |
| $10^{-7}$      | 35.60            | -                  | 15.70   |

\*ND=not done

The above experiment was repeated by further diluting the virus (from  $10^{-6}$  to  $10^{-12}$ ). The results are shown below.

| Virus dilution | Initial cT value of the virus dilution | Extent of diarrhea | cT value in mucosal samples from inoculated piglets |
|----------------|--|--------------------|---|
| $10^{-6}$      | 33.65                                  | +                  | 15.52   |
| $10^{-7}$      | 37.83                                  | ++                 | 15.52   |
| $10^{-8}$      | -                                      | +                  | 16.03   |
| $10^{-9}$      | -                                      | -                  | 30.29   |
| $10^{-10}$     | -                                      | -                  | -   |
| $10^{-11}$     | -                                      | -                  | -   |
| $10^{-12}$     | -                                      | -                  | -   |

**Update: 10-16-13**

PI: Sagar Goyal

**Infectious dose of PEDv:** To determine the infectious of PEDv, we conducted the following experiment. Serial 10-fold dilutions of PEDv (clarified homogenate of intestinal mucosa from infected piglet) were prepared in PBS and were tested by RT-PCR. The cT values are given in column 2 of Table 1. Column 3 shows the severity of diarrhea caused by different virus dilutions. Two days after virus inoculation, the pigs were killed, their intestinal mucosa collected, and tested for PEDv by PCR. cT values of this RT-PCR (in mucosa of inoculated piglets) are shown in column 4. The results indicate that PEDv is highly infectious and that the  $MID_{50}$  is very low.

| Virus dilution   | Initial cT value | Extent of diarrhea | cT value in mucosal samples from inoculated piglets |
|------------------|------------------|--------------------|---|
| Undiluted        | 16.39            | ND*                | ND  |
| 10 <sup>-1</sup> | 17.73            | ND                 | ND  |
| 10 <sup>-2</sup> | 20.53            | ++                 | 17.24   |
| 10 <sup>-3</sup> | 23.55            | ++                 | 16.92   |
| 10 <sup>-4</sup> | 27.04            | ++                 | 15.32   |
| 10 <sup>-5</sup> | 29.94            | +                  | 17.10   |
| 10 <sup>-6</sup> | 32.06            | +                  | 16.02   |
| 10 <sup>-7</sup> | 35.60            | -                  | 15.70   |

\*ND=not done

#### Update: 10-3-12

PI: Goyal

To confirm the results of RT-PCR, we have conducted bioassays (in 10-day-old piglets) for a selected number of samples. We will continue bioassays until we have tested all samples.

**Survival of PEDv in slurry:** At -20°C, PEDv RNA was detectable for 28 days and so was TGEv RNA. The slurry samples (spiked with PEDv) were taken at various time points (0, 1, 3, 7, 14, and 28 days) after storage at -20°C and were inoculated orally (intra-gastrically) in 10-day-old PED-negative piglets. Un-inoculated piglet served as a negative control. **Results of bioassay:** Samples taken at 0, 3, and 7 days produced diarrhea in inoculated piglets. Samples taken at 14 and 28 days did not produce appreciable diarrhea. All pigs were euthanized and their intestinal samples were tested by RT-PCR to determine the presence of PEDv. Intestinal samples from all pigs inoculated with samples from 0, 3, 7, 14, and 28 days at -20°C were *positive by RT-PCR* indicating that the virus did survive in slurry for 28 days at -20°C and that results obtained with RT-PCR matched those of bioassay. Intestinal sample from the un-inoculated piglet was negative for PEDv by RT-PCR. In addition, intestinal samples from all piglets (inoculated and un-inoculated) were negative for TGEv when tested by RT-PCR. The rectal swabs collected from piglets before inoculation were all RT-PCR negative for PEDv and TGEv.

**Survival of PEDv in animal feed:** At room temperature, neither TGEv nor PEDv RNA was degraded in feed for 28 days. The feed samples (spiked with PEDv) were taken at various time points (0, 3, 7, 14, and 28 days) after storage at room temperature and were inoculated orally (intra-gastrically) in 10-day-old PED-negative piglets. Un-inoculated piglet served as a negative control. **Results of bioassay:** Sample taken at 0 day produced diarrhea in inoculated piglets but those taken at 7, 14 and 28 days did not produce appreciable diarrhea (the 3-day sample was not inoculated because we lost one pig to non-related causes). All pigs were euthanized and their intestinal samples were tested by RT-PCR to determine the presence of PEDv. Intestinal samples from all pigs inoculated with samples from 0, 7, 14, and 28 days were positive by RT-PCR indicating that the virus did survive in feed for 28 days. These results match those obtained with RT-PCR. Intestinal sample from the un-inoculated piglet was negative for PEDv by RT-PCR. In addition, intestinal samples from all piglets (inoculated and un-inoculated) were negative for TGEv when tested by RT-PCR. The rectal swabs collected from piglets before inoculation were all RT-PCR negative for PEDv and TGEv.

#### Update: 9-21-13

Note: The following results are based on RT-PCR data. Bioassays (to determine survival of the whole virus versus RNA only) will begin soon. RT-PCR detects viral RNA semi-quantitatively and does not differentiate between live and

inactivated virus. To determine if the virus survives a particular condition, we need to do bioassay in piglets because we do not currently have a consistent method for growth and titration of PEDv in vitro.

**Survival of PEDv in fresh feces:** Experiments have been conducted to determine PEDv survival in fresh feces at 40°C, 50°C, and 60°C at three different relative humidity (RH) levels of 30%, 50%, and 70%. Viral RNA was detected for 7, 3, and 3 days at 40°C, 50°C, and 60°C, respectively. So far, we have not seen any effect of RH on RNA detection except that at 70% RH, the RNA could be detected for 7 days at 40°C and 50°C but not at 60°C. Under all conditions, TGEv RNA was detectable for a similar or longer period of time than that of PEDv.

**Survival of PEDv in slurry:** At -20°C, PEDv RNA was detectable for 28 days and so was TGEv RNA. Similar results were obtained at room temperature (at 30% RH). Results of additional time points are not available yet.

**Survival of PEDv in drinking and recycled water:** Not done yet.

**Survival of PEDv in animal feed:** At room temperature, PEDv RNA was not degraded in feed for 28 days. The TGEv results are very similar to these results.

#### **Update: 9-2-13**

**Survival of PED in fresh feces:** Experiments have been conducted at 30% relative humidity (RH). At 40°C the nucleic acid of PED is not degraded for 7 days. At 50°C, PED RNA could be detected for at least 3 days. Results of additional time points are not available yet. These results are based on RT-PCR data. Bioassays (to determine survival of the whole virus versus RNA only) will begin soon. The TGE survival results are very similar indicating that TGE may serve as surrogate for PED.

**Survival of PED in slurry:** At -20°C, PED RNA was detectable for 7 days. Similar results were obtained at room temperature (at 30% RH). Results of additional time points are not available yet. These results are based on RT-PCR data. Bioassays (to determine survival of the whole virus versus RNA only) will begin soon. The TGE results are very similar. Additional samples will be collected and tested at 14 and 28 days.

**Survival of PED in drinking and recycled water:** Not done yet.

**Survival of PED in animal feed:** At room temperature, PED RNA was not degraded in feed for 14 days. Results of additional time points are not available yet. These results are based on RT-PCR data. Bioassays (to determine survival of the whole virus) will begin soon. The TGE results are very similar.

Quick Take

PEDV is a persistent virus, so determining its environmental limits is important for control efforts.

#### **Update: 8-21-13**

##### Environmental stability of PED

*Virus stock:* A stock of virus has been prepared that will be used in all experiments. Small intestines collected from PED-infected piglets were cut into 5-10 cm long sections and washed with sterile PBS (pH 7.2). Mucosa was scraped with a glass slide and homogenized in sterile PBS. Following centrifugation at 2000xg for 15 min, the supernatant was collected, aliquoted, and stored at -80°C. The presence of PED in the supernatant was confirmed by real time RT-PCR using S gene primers (cT value =17.16 ). For comparative purposes, a stock of TGEV (transmissible gastroenteritis virus) was prepared by growing the virus in ST (swine testicle) cells.

*Survival in slurry:* Samples of slurry that were negative for PED and TGEV by rRT-PCR were aliquoted in 3.5 g amounts in sterile vials. Set A was spiked with 100 µL of 3X antibiotics and 0.5 mL of PEDv stock. Set B was spiked with 0.5mL of TGEV. The vials were placed at -20°C. Three vials of each set were removed at 0 and 1 day of incubation followed by the addition of 5 mL of a sterile solution of 3% beef extract-0.05M glycine (pH 7.5). After mixing, the vials were centrifuged at 1,200xg for 10 min. The three supernatants obtained were pooled from each set of samples (at each time point). Serial 10-fold dilutions were prepared from each pool and tested for PED and TGEV by rRT-PCR and by inoculation in ST cells, respectively. The results of RT-PCR indicate that PED survives for one day at -20°C. Additional samples will be collected and tested at 3, 7, 14 and 28 days. These samples have been stored at -80°C pending IACUC approval for bioassays.

*Survival in feed:* Aliquots of animal feed (5g amounts) were placed in sterile vials. To each vial was added 10 mL of sterile PBS to achieve a slurry-like consistency. PED (set A) or TGEV (set B) was added @ 1mL per vial. After mixing well, the vials were stored at RT (approx. 25°C). Three tubes from each set were removed at 0 and 1 day. The virus was eluted by adding 5mL of 3% beef extract-0.05M glycine solution. After thorough mixing, the vials were centrifuged at 1,200xg for

10 min. Elutes from each of the 3 vials were pooled, serial dilutions of the pools were prepared, and tested by rRT-PCR (for PED) and by inoculation in ST cells (for TGEV). The results of RT-PCR indicate that PED survives in feed for one day. We plan to test samples at 3, 7, 14 and 28 days. All samples will be stored at -80°C for use in bioassays.