RESEARCH REPORT
SWINE HEALTH

Title: Bioassay for Porcine Epidemic Diarrhea Virus contaminated feed **– NPB #14-150**

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Industry Summary:

Within the past year, Porcine Epidemic Diarrhea Virus (PEDV) has emerged as a significant swine disease in the United States. This coronavirus continues to spread within the U.S. swine herd resulting in significant morbidity and mortality, especially in neonatal piglets. Rapid dissemination has occurred following the first detection of PEDV in the United States in May 2013. Retrospective investigations of several PEDV outbreaks have detected PEDV RT-PCR-positive feed leading to suggestions that contaminated feed ingredients may be a potential source of PEDV introduction into herds.

The present project was initiated to assess if feed testing positive for PEDV with RT-PCR was infectious to pigs. This study was part of a larger investigation into an outbreak of PEDV in a commercial swine production system. The feed used in the present study was from a farm that experienced its first PEDV outbreak in January 2014. Feed used during the outbreak tested positive for PEDV with an RT-PCR assay; however, RT-PCR only detects the presence of viral RNA and does not mean viable and infectious virus was present in the sample. In the on-farm case, the attending veterinarian froze aliquots of the suspect feed as a wet mash in an attempt to preserve viable virus. PEDV is extremely difficult to culture outside of a pig model; therefore, a bioassay was used to assess infectivity of the feed fed during the outbreak. The suspect feed was fed to PEDV susceptible pigs for one week, during which time the pigs, feed, and environment were repeatedly tested for PEDV.

Even though the feed was RT-PCR positive for PEDV during the study, environmental and rectal swabs collected daily during the study were negative for PEDV using RT-PCR. No clinical signs of disease were observed among the pigs during the bioassay. Microscopic examination of intestinal tissues collected from the piglets at the end of the study revealed no significant morphologic lesions. Therefore, the results of bioassay were not able demonstrate any evidence that the RT-PCR positive feed fed in the current study was infectious and capable of causing disease.

While we were not able to confirm the feed as a source of PEDV in the present case, the sensitivity of the bioassay may have been limited by the small number of pigs (n = 10) and the amount of feed the weanling age (from 10 to 20 days of age) pigs could consume during the trial period. Additionally, there was a 28 day lag from the time the feed was manufactured to the time of the initiation of the bioassay, a time period that may have decreased the viability of any virus that may have been present in the feed at the time of delivery to the farm. Nonetheless, the growing number of case reports implicating feed as a potential PEDV source indicates systematic, active surveillance of emergent porcine coronaviruses in feed and feed components is needed to overcome the inherent limitations of retrospective investigations.

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Keywords:
PEDV, Bioassay, Feed, PCR, infectivity

Scientific Abstract:

Retrospective investigation into an outbreak of PEDV in a commercial swine production system in January 2014 found feed testing PEDV-positive with RT-PCR. This finding indicated that contaminated feed may have been a potential source of PEDV introduction into the herd. Since RT-PCR only detects the presence of viral RNA and it does not mean viable and infectious virus was present in the sample, this project was initiated as a timely follow-up to assess if the suspect feed (testing positive for PEDV with RT-PCR) was infectious to pigs. At the time of the outbreak, the attending veterinarian froze aliquots of the suspect feed as a wet mash and in the originally manufactured, pelleted form in an attempt to preserve viable virus. The suspect feed aliquots were fed to PEDV susceptible young pigs (starting at 14 d of age) for one week, during which the pigs, feed, and environment were repeatedly tested for PEDV.

The feed tested weakly PEDV positive with RT-PCR during the study (mean Ct =36.5). Pigs showed no clinical signs of disease during the bioassay. Environmental and rectal swabs collected daily during the study were negative for PEDV using RT-PCR. Microscopic examination of intestinal tissues collected from the piglets at the end of the study revealed no significant morphologic lesions. The result of the bioassay provided no evidence that that the feed contained infectious PEDV and was capable of causing disease.

While we were not able to identify infectious PEDV with the present bioassay study, the inherent limitations of a retrospective bioassay make it impossible to rule out feed as a potential source of virus for this outbreak. Molecular sequencing and epidemiological data from this case may provide more insight to potential routes of PEDV introduction. Nonetheless, the growing number of case reports implicating feed as a potential PEDV source indicates systematic, active surveillance of emergent porcine coronaviruses in feed and feed components is needed to overcome the limitations of retrospective investigations.

Introduction:

Within the past year, Porcine Epidemic Diarrhea Virus (PEDV) has emerged in the United States. This highly contagious and deadly coronavirus has continued to spread within the U.S. swine herd following the first report in May 2013. The introduction of PEDV into the U.S. swine herd has resulted in significant morbidity and mortality, especially among neonatal piglets. The fiscal damage that these viral infections have inflicted on the nation’s pork industry has been severe.

This enteric pathogen is believed to be primarily spread between animals through fecal-oral transmission. Pigs acutely infected with PEDV shed tremendous quantities of virus for several days following infection making it very difficult to control the spread of the virus. Rapid dissemination of PEDV across 23 states has occurred even in the face of heightened awareness and increased biosecurity. Retrospective investigations of several PEDV outbreaks have found PEDV-positive feed using RT-PCR assays, leading to suggestions that contaminated feed or feed ingredients are potential sources of PEDV.

PEDV is difficult to culture outside of pig; therefore, RT-PCR assays are currently the only tests available to pork producers and swine veterinarians to detect PEDV. Because RT-PCR only detects the viral RNA, a positive RT-PCR result only indicates detection of PEDV viral nucleic acid, which designates the presence PEDV genetic material, but does not mean that viable and infectious virus is present in the sample. The lack of agreement between RT-PCR results and biological infectivity makes test interpretation very difficult.

Objectives:

The goal of the project was to assess if feed containing RNA (RT-PCR positive) from porcine epidemic diarrhea virus (PEDV) is a means by which the virus can be spread. The present study was part of an investigation into an ongoing PEDV outbreak in a swine production system. Investigation of the outbreak identified PEDV contaminated feed as a likely route of viral introduction and infection. In the present study, we sought to test the feed introduction hypothesis by performing a bioassay (feeding the suspected feed, which is RT-PCR positive for PEDV, to naive animals and recording the outcome).

Materials & Methods:

Ten, 10-day-old pigs, were obtained from a commercial sow herd. The sows in the source herd were negative for PEDV during routine surveillance prior to the trial. Fecal swabs, collected from the pigs at the source herd prior to the study, were also negative for PEDV. Environmental sampling, using Swiffer cleaning cloths, of the research facility prior to pig entry was negative for PEDV with rRT-PCR. RT-PCR testing of rectal swabs collected from each pig upon entry to the facility and environmental swabs of the transportation equipment were negative for PEDV.

The pigs were fed a commercial swine feed negative for PEDV for 108 hours following their arrival at the research facility. Feed was mixed with water to make a gruel to improve feed consumption. Rectal swabs were collected from the pigs on a daily basis. Feed samples and environmental swabs were also collected on a daily basis. All pigs were observed consuming feed a minimum of three times per day throughout the study.

Feed, sampled from the production site, and suspected as a PEDV introduction source for the infected farm was used in this study. At the time the PEDV outbreak was recognized on the farm, the attending veterinarian for the herd collected the pelleted feed from the farm. These pellets tested positive for PEDV using RT-PCR. Aliquots of collected feed were aseptically mixed with sterile water to make a mash. These moistened, mash aliquots were stored at $-20^{o}C$ until the bioassay could be performed. Following the acclimation period, the pigs were provided ad libitum access to the RT-PCR positive mash along with dry pellets from the same lot, for 7 days and observed for clinical signs of PEDV. Feed samples, environmental swabs, and rectal swabs were collected each day of the study. After 7 days, the pigs were euthanized and intestinal tissues were submitted for diagnostic testing.

Results:

The environment, feed, and pigs were PEDV negative using RT-PCR prior to the study and during the 108 hour acclimation period.

As shown in Table 1, the feed tested weakly PEDV positive with RT-PCR during the 7 day study (mean Ct =36.5). No clinical signs of disease were observed in the pigs during the bioassay. Environmental and rectal swabs collected daily during the study were negative for PEDV using RT-PCR. Microscopic examination of intestinal tissues collected from the piglets at the end of the study revealed no significant morphologic lesions.

TABLE 1. Results of rRT-PCR testing for the presence of PEDV conducted during the bioassay.			
	Feed	Pigs	Environment
Pre-entry	Negative (Ct >42)	All 10 negative (Ct>42)	Negative (Ct >42)
Entry	Negative (Ct >42)	All 10 negative (Ct >42)	Negative (Ct >42)
Day 0	Negative (Ct >42)	All 10 negative (Ct >42)	Negative (Ct >42)
Day 1	Negative (Ct >42)	All 10 negative (Ct >42)	Negative (Ct >42)
Day 2	Negative (Ct >42)	All 10 negative (Ct >42)	Negative (Ct >42)
Day 3	Negative (Ct >42)	All 10 negative (Ct >42)	Negative (Ct >42)
Day 4	Ct =35	All 10 negative (Ct >42)	Negative (Ct >42)
Day 5	Ct =37	All 10 negative (Ct >42)	Negative (Ct >42)
Day 6	Ct =35	All 10 negative (Ct >42)	Negative (Ct >42)
Day 7	Ct =39	All 10 negative (Ct >42)	Negative (Ct >42)
Day 8	Ct =37	All 10 negative (Ct >42)	Negative (Ct >42)
Day 9	Ct =42	All 10 negative (Ct >42)	Negative (Ct >42)
Day 10	Ct =31	All 10 negative (Ct >42)	Negative (Ct >42)

Discussion:

The results of this study could not demonstrate that the feed contained infectious PEDV which was capable of causing disease. While the present study was not able to confirm the feed as a source of PEDV, feed cannot be ruled out as a source of the outbreak. The sensitivity of the bioassay was limited by the amount of feed the individual pigs and the small number of pigs collectively could consume during the trial period. In a field setting where there are thousands of pigs consuming tons of feed, it is conceivable that a very small amount of infectious PEDV would be capable of initiating an outbreak that would spread through the population of susceptible animals. In addition, the present study may have been hindered by the 28 day lag from the time the feed was manufactured and the initiation of the bioassay. Other investigators have demonstrated short-term survivability of PEDV in feedstuffs. The time lag likely decreased the viability of any infectious PEDV that was present at the time of delivery to the farm. These limitations are common among all retrospective investigations.

Ultimately, the growing number of case reports implicating feed as a potential PEDV source indicates systematic, active surveillance of emergent porcine coronaviruses in feed and feed components is needed to overcome the inherent limitations of retrospective investigations. Ultimately, establishing the ecology and epidemiology of PEDV is critical to swine production and national food security.