RESEARCH REPORT
SWINE HEALTH

Title: Infectivity of swine manure from pits on sow farms at varying lengths of time post infection with porcine epidemic diarrhea virus (PEDV)", NPB #14-276

Investigator: Steve Tousignant, DVM, PhD Candidate
(stous@swinevetcenter.com) 952-393-5535

Institution: Swine Vet Center P.A., Saint Peter, MN

Date submitted: 5/27/2016

Industry Summary:
This project was designed to test manure from manure lagoons on sow farms across the midwest for the presence of viable PEDV in an effort to understand the risk of transmitting this virus during pit pumping. Of the pits we tested, we found that only one lagoon had evidence of infectious virus present, and this farm was at that time weaning PED positive piglets. These data continue to emphasize the need for proper biosecurity measures and planning during the pit pumping, especially when dealing with known PED positive sow farms.

In this study, we sampled 5 manure lagoon from barns in Iowa, Nebraska and North Dakota. At each site, manure was sampled by tossing a small bucket attached to a cord into the lagoon at three different locations. The manure samples were tested individually for PEDV at the University of Minnesota Veterinary Diagnostic lab by polymerase chain reaction (PCR) and also tested by swine bio-assay for presence of live virus.

All manure samples were positive for PED by PCR. One case also tested positive for Swine Delta Coronavirus on a herd that was co-infected with both viruses. We found that on average, lagoons had a PCR cycle time of 30, indicating a relatively high amount of viral genetic material. When tested by swine bio assay, one barn that was presently shedding PED (weaning PED positive piglest) was positive.

In the swine bio-assay, 20 mL of manure from one site was administered via a stomach tube to a single pig and was observed for 3 days. At the end of the study the pig was taken to the diagnostic laboratory and infection was confirmed by the diagnosticians there.

In conclusion, of the 5 lagoons sampled in this project, only one had evidence of viable virus. Therefore, it is recommended to continue to be very diligent regarding biosecurity and sequencing of manure pumping equipment. It is important to note, that it is possible for to have missed live PEDV in several of the lagoons simply due to issues of sample size. It is hopeful that careful planning and good communication may help minimize the spread of PEDV during future manure pumping events.

Keywords: PEDV, bio-assay, manure, pumping

Abstract:

Since introduction into the US swine herd, PEDV has spread rapidly across swine producing regions causing dramatic production losses. This study was designed to test 5 manure lagoons for the presence of live PEDV using swine bio-assay. In this study, only one site was positive for live virus (by bioassay) whereas the 4 others were negative for live virus. The one positive site was actively shedding PEDV as indicated by the testing of weaned pigs. These observations reinforce the importance of biosecurity, especially with pit pumping crews where it is strongly advised to pump negative sites first, and work toward positive sites while paying particular care with those sites most recently infected with PEDV.

Introduction:

Porcine epidemic diarrhea virus (PEDV) was first diagnosed in the United States (US) swine herd in May 20131. Since then it has spread rapidly, and near by the end of June 2013, the virus had infected nearly 50% of 739 participating sow herds in the Swine Health Monitoring Project. Early case reports from 4 farms included dramatic accounts of 90 – 95% mortality in suckling pigs characterized by profuse watery diarrhea containing undigested milk, vomiting, and dehydration. Due to the rapid dissemination of this virus in the US swine herd, immediate investigations into the roles of transportation, aerosol transmission and feed were started quickly. Each study concluded the potential for transmission by its respective route.

Early studies have shown PEDV to be stable in fecal material and slurry for varying amounts of time from 14 days to more than 1 month, depending on temperature (Goyal #13-215: University of Minnesota Environmental stability of PED (porcine epidemic diarrhea) virus. National Pork Board). Additionally, a previous study showed that viable PED virus can be found in the deep manure pits under grow-finish barns between 4 and 6 months after PED was known to be on the site (Tousignant #14-246: Swine Vet Center, Infectivity of swine manure from pits at varying lengths of time post infection with Porcine Epidemic Diarrhea (PED) virus). There is still a growing concern that the pumping and transportation of swine manure from lagoons and subsequent application to agricultural fields may be a significant risk factor for transmission of viable PEDV in the swine industry.

Objectives:

The objective of this study was to sample manure from sow farms that were at varying lengths of time post-PEDV infection and test for the presence of viable virus by swine bio-assay.

Materials and Methods:

Sample site selection – Sow farms were selected during a time period in late June 2015 through early May 2016 based on documented history of PEDV infection on site.

Sample collection – Samples were collected using a small bucket attached to a 30’length of cord. At each site, samples were drawn from the lagoon at various locations around the perimeter including the shallow area near the effluent pipe, the deep area near the effluent pipe, and the deep area on the far end from the effluent pipe. Individual samples were placed into sterile specimen jars. New, clean buckets and cord were used at each sampling site. Samples were stored under refrigeration for no more than 2 days before use, and were frozen at -20 degrees Celsius if storage longer than 2 days was required.

Sample Assays – Aliquots of each sample were sent to the University of Minnesota Veterinary Diagnostic Laboratory for PEDV Polymerase Chain Reaction (PCR) testing.

Swine bio-assay – Samples that tested positive for PEDV by PCR, were then tested by swine bio-assay. 10 day old barrows from a PRRS, and PED negative (naïve) sow flow were obtained for this study. Pigs were housed individually, bedded with pine shavings and fed a standard pelleted nursery ration without products of swine origin ad libitum. Pigs were randomly assigned to a sample site for which they were to be inoculated with. A 20 ml sample of undiluted manure slurry was administered to the pig via a 14 French gastric tube/urinary catheter (Bard Medical, Covington, GA, USA) attached to a 60cc syringe. Pigs were manually restrained, and the tube was slowly passed into the back of the oral cavity allowing the pig to swallow the tube. To ensure the tube had not been inadvertently passed into the trachea, negative pressure was applied to the syringe. If air passed into the syringe, the tube was removed from the mouth of the pig, and the process was started again. If no air was drawn back, the sample was slowly administered to the pig over 5-10 seconds. The tube was pinched closed and withdrawn slowly to prevent regurgitation and the pig was returned to the individual pen. Pigs were observed for a period of 3 days and then transported to the University of Minnesota Veterinary Diagnostic Laboratory for necropsy, intestinal PCR, histopathology and Immunohistochemistry.

Four negative controls were used during the study where pigs were inoculated with manure from sites confirmed to be PEDV negative. Two positive controls were conducted where pigs were inoculated with known PEDV positive intestinal homogenate obtained from a sow farm using it to expose and acclimatize newly arrived replacement gilts to PEDV present on that farm.

Results:

Bio-assay

All samples were positive by PCR, and therefore tested by bioassay. One site tested positive eby Bioassay as indicated by a lower PCR CT value in the intestines (14.76) than in the inoculum (29.49), as well as indications of infection noted on histopathology including severe villous tip necrosis and immunohistochemistry positive. Clinical signs of diarrhea were not observed in this piglet. All other samples (n=27) were negative.

Discussion:

There are important limitations to this study which should be acknowledged. First, only 60 ml (20 mL x 3 samples per lagoon) of manure were tested by bio-assay in a pit that may have contained millions gallons of liquid manure. Additionally, samples were collected by tossing a bucket attached to a cord into the lagoon and drawing it back out. These limited samples may not accurately reflect the true status of the manure from these sites. Recent work might suggest an effect of depth on the viability of manure in lagoons through an arguably more precise sampling method.

Ultimately these data help reinforce the ongoing attention to established protocols for pumping swine manure. Generally, it is advised that sites that are free from various diseases are pumped first, followed by sites that are endemically, or more recently infected with diseases (such as PEDV). These data might also suggest pumping sites that have been most recently infected with PEDV last. Additionally, proper hygiene is always recommended between sites, including washing and disinfecting vehicles, tools and equipment. If additional days of down-time between sites are practical, it is recommended. Finally, communication with neighboring sites is critical to ensure all efforts have been explored to minimize risk of moving potentially infectious manure around naïve or negative sites.