# Non-Ruminant Session

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# Porcine Epidemic Diarrhea Virus in Feed – What We Know and What We Don't Know

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## Summary

Until April 2013, Porcine Epidemic Diarrhea virus (PEDV) was not present in the United States or Canada, but it was common in Europe and Asia. Infection with PEDV causes acute outbreaks of watery diarrhea within 24 hours of infection. Morbidity approaches 100% in young (< 7 day old) pigs, but it is variable in older pigs with minimal clinical signs in adult pigs. Similarly, mortality in young pigs ranges between 50 to 100% when infecting naïve herds with minimum or no mortality in adult pigs. Since its introduction in the US, PEDV may have caused the loss of 7 million young pigs. Transmission of PEDV in feed or feed ingredients is suspected based on several observations: 1) introduction of the virus in the United States of America and its rapid spread to Canada, Mexico, Peru, Colombia, and the Dominican Republic after ruling out other transmission routes, 2) transmission of PEDV in Canada was initially associated with feed ingredients of porcine origin, 3) other cases of PEDV transmission between herds point to several nursery barns infected within a short window of time and sharing a similar source of feed, but all filled with pigs from negative sow herds. These observations lead veterinarians to the hypothesis of in feed transmission of PEDV. Since then, feed ingredients of porcine origin have been removed from many feeding programs as precautionary measures. These measures are disruptive and increase cost without clear estimate of the overall reduction in risk of PEDV transmission. Therefore, 2 distinct, but complementary projects are under execution at the University of Minnesota along with other projects to provide solutions to the problem. The objective of the first project is to measure PEDV inactivation on various conditions (temperature, humidity, pH, and water activity). The objective of the second project is to estimate the risk of disease transmission when including ingredients of porcine origin in swine diets. Preliminary data suggest distinct conditions of processing feed ingredients of porcine origin and consequently a different risk for ingredients in each process. Therefore, sound risk mitigating strategies are different depending on the feed ingredient and processes involved in production and commercialization of the ingredients. Pork producers, veterinarians, and swine nutritionists should understand these differences to make adequate decisions in swine feeding programs.

## Introduction

The current outbreak of Porcine Epidemic Diarrhea (PED) is causing an approximate 11% reduction in availability of market hogs in the US, but also other countries such as Mexico, Colombia, Japan, and Korea have been affected. In May 2014, Rabobank estimated that along with the outbreak of African Swine Fever, global pork production will decline 6-7% in 2014. The 11% reduction in availability of market hogs in the US is considered the most relevant reduction in the last 30 years (Verheul, 2014). When Porcine Epidemic Diarrhea Virus (PEDV) infects a naïve herd, it may cause mortality in young pigs between 50 and 100% (Saif et al., 2012). Therefore, reducing the impact of PEDV in pork production in the US is a current priority, which relies on finding and eliminating all possible routes of disease transmission. One possible route of disease transmission is via feed ingredients and complete diets. This has caused diet modifications and increment in feed cost. Therefore, risk mitigating strategies require data and

comprehensive analysis of the impact of the changes incurred. The objective of this literature review is to offer an overview of the disease characteristics, early evidence of disease transmission in feed, and describe current projects to find solutions to the PEDV feed transmission issue.

**General aspects of Porcine Epidemic Diarrhea**

***Etiology:*** Porcine Epidemic Diarrhea Virus belongs to the virus family Coronaviridiae, crown like envelope that gives its distinct name, along with the virus of Transmissible Gastro-Enteritis (TGE) and Deltacoronavirus (Saif et al., 2012) in pigs.

***Epidemiology:*** Prior to 2013, PEDV was common in Europe and Asia. However, the strain of virus causing the disease in Europe appears less virulent than the strain currently affecting North America (Saif et al., 2012; Jung et al., 2014). Therefore, the disease in Europe has been of less economic importance. In Asia (China and Korea) the disease has been endemic causing diarrhea in young pigs. Direct fecal-oral transmission is the major route of PEDV transmission. Indirect transmission is also possible from contamination of fomites, boots, birds, movement of infected animals among herds, and feed and pig transport trucks.

***Pathogenesis:*** the strain of virus affecting North America (strain PC21A) was characterized as highly pathogenic (Jung et al., 2014). Macroscopic lesions occur from duodenum to colon with intestine walls becoming extremely thin. Microscopic lesions indicate severe atrophic jejunitis with loss of villi and increment in crypt size.

***Clinical signs:*** watery diarrhea is the most common clinical sign of PEDV infection in piglets, but events of vomit are also observed (Jung et al., 2014; Dee et al., 2014). Diarrhea develops soon (24 to 48 h) post infection regardless of the inoculum dose (6.8 to 9.0 genome equivalents) indicating that a very small amount of virus is required to infect and cause disease. Pigs become dehydrated within a short period (< 120 h) post infection given the profound water loss in feces. This diarrhea occurs due to destruction of the intestinal wall (from duodenum to colon) with severe jejunitis, signs observed also with the Asian virus strains (Kim and Chae, 2000). Clinical signs in young pigs are more acute with mortality ranging from 50 to 100%, while sows may present minimal signs of discomfort or transient diarrhea. Disease is "self-limiting" once lactogenic immunity is developed and new litters are infected mostly from gilts that have not developed immunity (Martely et al., 2008).

***Diagnosis:*** PEDV can be identified directly by immunohistochemistry in samples of jejunum and ileum of affected pigs, by virus isolation in Vero Cells, or direct electron microscopy of virus particles in feces. Quantitative polymerase chain reaction (qPCR) can be used as means of detecting virus genetic material (RNA) in fecal material along with swabs of surfaces, fomites, and feed. Clearly, infection with PEDV must be differentiated from TGE, neonatal colibacilosis, and rotavirus infections. Direct techniques such as virus isolation and immunohistochemistry have limitations on the number of samples tested and sensitivity of the test, while use of qPCR allows for virus quantification. Virus isolation has limited sensitivity generating many false negatives because most field strains of virus do not grow on laboratory conditions. Use of qPCR does not allow differentiating viable from non-viable virus particles. Therefore, infecting susceptible pigs (bioassay) is the final method to determine infectivity of feed and feed ingredients.

## Transmission of PEDV in feed

Transmission of PEDV in feed and feed ingredients is suspected due to various observations. In Canada there were outbreaks of PED in multiple nursery farms that sourced weaning pigs from multiple negative sow farms indicating PEDV source other than vertical transmission. All these farms shared the same source of pre-starter and starter diets and that sparked a recall of feed from Grand Valley Fortifiers. Grand Valley Fortifiers and farm veterinarians performed PCR tests on 55 truck deliveries that resulted negative, ruling out the transmission from contaminated trucks. Therefore, the investigation continued testing 76 samples of nursery pig diets and 6 samples of Spray Dried Porcine Plasma (SDPP). Samples of feed (3/76) and SDPP (5/6) tested positive by PCR. The PCR test confirms presence of virus, but not if the virus is capable of infecting a susceptible host. Therefore, the Canadian Food Inspection Agency (CFIA) tested virus infectivity by feeding susceptible pigs with the diets and SDPP under investigation.

Similarly, in the US, several farms sourcing weaning pigs from different negative sow farms also broke with PED without explanation from any other route of transmission. Veterinary investigations and sampling pointed to an association between contaminated feed or feed bins and the PEDV outbreak on those farms (Dee et al., 2014). Specifically, in a case in Minnesota and Iowa, the virus genetic material was detected in feed and feed bins using synthetic woven rollers to capture virus (Dee et al., 2014). Then, the suspected feed and samples of feed bins were fed to susceptible pigs, which reproduced the disease. This observation clearly suggests that feed and feed equipment can be contaminated and a source of virus transmission. However, this evidence does not demonstrate if the source of virus if from contaminated feed mill or simply contamination from the environment close to the infected farm.

Collection of feed samples for analysis of PEDV requires the use of aseptic techniques to avoid collection of environmental PEDV. The Applied Swine Nutrition Group of Kansas State University has developed a simple procedure available at the American Association of Swine Veterinarians website for sample collection for PEDV testing. Briefly, feed (1 to 2 lb) samples can be scooped into sterile whirl-pak bags using sterile utensils (AFCO, 2000). Because positive feed samples can be isolated within a batch of feed, multiples samples of the same batch are necessary to reduce chances of false negative results. These procedures are not different from sampling feed in cases of *Salmonella* infection. Samples could be pooled to reduce cost of feed testing. The Veterinary Diagnostic Laboratory of the University of Minnesota offers services for qPCR and bioassay of feed samples.

## Processes of feed that may inactivate the virus

Virus transmission in feed and feed ingredients can be prevented by inactivating the virus in several processes. However, inactivation of the virus will depend on the combination of variables such as heat applied, duration, and matrix nutrient that contains the virus. There is a wide range in time and temperature combinations among common feed processing methods such as pelleting, extrusion, spray-drying, and rendering (Table 1). The greatest processing temperatures are applied in rendering (115 to 145 °C) with extended retention (45 to 90 min), while during pelleting feeds are heated at lower temperatures (65 to 95 °C) and shorter retention times (30 to 90 seconds).

Evaluating the conditions of heat and temperature at which PEDV is inactivated is part of a project funded to Dr. Sagar Goyal at University of Minnesota from the National Pork Board. Results of this project are not complete at this time, but will be useful data for a second project that will measure the risk of PEDV survival and transmission in feed ingredients of porcine origin

(Table 2). Specific pelleting conditions that inactivate PEDV will be studied at Kansas State University.

Finally, after feed processing and potential virus inactivation, the feed and feed ingredients should be handled to avoid cross contamination. Dr. Laura Greiner of the Carthage Innovative Swine Solution will investigate the risk of feed mill contamination and this project will be complementary to investigating the role of birds as vectors of PEDV transmission. Therefore, biosecurity protocols adapted to PEDV inactivating conditions are expected to be adjusted to avoid virus transmission from cross-contamination. The final reports of these projects were not available at the time of writing this manuscript. Therefore, readers are encouraged to visit the National Pork Board website for updates on the progress of these projects and final reports due late fall 2014.

**Table 1.** Time and temperature variables for common feed processing.

| Process | Heat | Pressure | Time | pH |
|---|---|---|---|---|
| Pelleting | 65-95 °C | Steam pressure of 30 PSIG<br>Die pressure 75-600 kg/cm2 | 30-90 s | No change |
| Extrusion | Stream: 70-90 °C<br>Chamber: 100-150 °C | Steam: 345 kPa | 10-90 s | No Change |
| Spray-drying | Inlet: 240 °C<br>Outlet: 90 °C | No change | Not clear | No change |
| Rendering | Cooking 115-145 °C | If pressure cocker is used | 40-90 min | No change |

**Table 2.** Projects currently funded by the NPB.

| Project Title | Institution; PI |
|---|---|
| Feasibility of viability of PCR and ex-vivo bioassay to detect viable PED virus in feed | University of Minnesota; Torremorell |
| Interventions to control PEDV (porcine epidemic diarrhea virus) in feed and feed ingredients | University of Minnesota; Goyal |
| Evaluation of the risk of a feed mill being contaminated with PEDV or SdCV | Carthage Innovative Swine Solutions, LLC; Greiner |
| Determining the impact of conditioning time and temperature in pelleted diets on PEDV survivability in complete swine diets | Kansas State University; Jones |
| A survey of *Sturnus vulgaris* (common Starlings) near swine premises to determine the potential roles as a vector of porcine epidemic diarrhea virus and swine deltacoronavirus | AMVC, LLC; Thomas |
| Risk assessment of feed ingredients of porcine origin as vehicles for transmission of Porcine Epidemic Diarrhea virus (PEDV) | University of Minnesota; Davies |

## Conclusion

The impact of PEDV on pork production in North America is already noticed on the number of pigs harvested in 2013 and predictions for 2014.Therefore, measures that mitigate PEDV transmission are necessary. The role of feed and feed ingredients on PEDV transmission is yet

to be determined, but early evidence point towards feed as the initial source of transboundary introduction and source of PEDV for introduction between farms.

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