10^3, 10^2, and 10^1 TCID50/g corresponding to a Ct of 27, 30, 33, and 37, respectively) and Ct increased linearly ($P < 0.01$, R^2 0.98) as PEDv dose decreased. Every 1 log reduction in PEDv concentration resulted in an increase in 3.4 ± 0.21 Ct in feed with detectable PEDv RNA. When the PEDv was added to the feed, an increase of 9.6 ± 0.4 SEM Ct was observed compared with the tissue culture PEDv concentration for those feed samples that had detectable PEDv RNA. When the supernatant was used to inoculate 10-d-old pigs, fecal sample Ct ranged from 16 to 27 on d 4 and 6 after inoculation for pigs inoculated with the 4 highest feed doses. No detectable PEDv RNA (Ct > 45) was noted in pigs inoculated with the other doses. Infection with PEDv was further confirmed in pigs from these 4 doses by histopathology and PEDv specific immunohistochemistry. In conclusion, these data suggest that PEDv infectivity was correlated with a positive feed PEDv PCR analysis. The minimum infective dose of PEDv in a feed matrix was demonstrated to be 5.6×10^1 TCID50/g and had an equivalent feed PCR Ct of 37. Overall, these data confirm that feed can be a vehicle for PEDv transfer and that a Ct of 37 can lead to infectivity in 10-d-old pigs.

Key Words: bioassay, feed, minimum infectious dose, Porcine epidemic diarrhea virus

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161 **Recent research into feed processing and biosafety.**

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The U.S. feed industry is under increased regulatory scrutiny as the Food Safety Modernization Act (FSMA) will soon require feed mills to have food safety plans with hazard analysis and controls. In addition to traditional physical and chemical hazards, biological hazards such as *Salmonella* and Porcine epidemic diarrhea virus (PEDv) have also been associated with feed. *Salmonella* deaths from shell eggs in 2010 were traced to unsanitary conditions in layer houses and a feed mill, and *Salmonella* illnesses in humans were traced to contaminated dry dog food in 2012. More recently, feed has been proven to be 1 of the many vectors of PEDv transmission in the swine industry. Fortunately, there are solutions to minimize these potential feed pathogens. Interventional strategies to minimize biological hazards in feed can be categorized as thermal or nonthermal. Pathogen mitigation by thermal processing is relatively practical, but the time and temperature relationship required is dependent upon the pathogen, dose, and matrix. Additionally, steps must be taken to prevent recontamination of animal feed during cooling, load out, and transportation because thermal inactivation of pathogens occurs at a point in time, but has no sustained effects to prevent recontamination. Nonthermal mitigation strategies, such as the inclusion of chemical additives, typically retain some mitigation characteristics to some level to prevent recontamination. However, chemical mitigants may have drawbacks associated with worker safety, consumer acceptance, or regulatory approval. For example, commercial formaldehyde has been shown to be effective at mitigating PEDv in swine feed and protein meals, but it is only approved for use in animal feed for *Salmonella* control. Again, the effectiveness of various chemical mitigants depends upon matrix and inclusion level. The feed industry has advanced its understanding of feed biosafety in recent years. Nevertheless, additional research is needed to refine pathogen mitigation strategies to maximize effectiveness while simultaneously increasing practicality of implementation.

Key Words: biosafety, feed, pathogen, Porcine epidemic diarrhea virus, *Salmonella*

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162 **An epidemiological investigation of porcine-origin feed ingredients and the occurrence of porcine epidemic diarrhea on midwestern U.S. pork farms.**

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Porcine epidemic diarrhea virus (PEDv) is a virus in the family Coronaviridae, confirmed for the first time in the United States on May 16, 2013. An investigation of the Canadian outbreak by the Canadian government identified an empirical association between feeding porcine blood plasma (subsequently found to be PCR positive for PEDv genetic material) and occurrence of the disease. To investigate the association between exposure to porcine-origin feed ingredients and the occurrence of PEDv, an epidemiological investigation of midwestern U.S. pork farms was undertaken in May 2014. Feed delivery data related to pig farming clients confirmed as being infected with PEDv (case farms) were obtained from a commercial feed manufacturer's information system. All deliveries of feed in the 2 wk prior to each PEDv outbreak that contained either spray dried plasma protein (SDPP), granulated red-blood cells (SDBC), choice white grease (CWG), or hydrolyzed porcine proteins (HPEP) were identified. The batching number for each feed delivery was then back-traced through the feed manufacturer's information system to identify the lot-level detail for each of the ingredients listed above that were included in the deliveries. This list of risk-associated lot numbers was then forward-traced to identify the complete list of clients to whom feed deliveries were made that included these same ingredient lot numbers but were not infected with PEDv (control farms). The risk of PEDv that was associated with exposure to specific lots of porcine-origin feed ingredients was then determined by estimating the odd's ratio (OR) for each ingredient (at both the ingredient-level and the lot-level). Forty-three cases and 418 controls were included in the study, with the most important limiting factor being the availability of ingredient data at the lot-level. Two sources of SDPP (5 and 6 lots, respectively), and 1 source each of SDBC (3 lots), CWG (10 lots), and HPEP (1 lot) were included in the analysis of risk. No ingredients were positively associated (OR > 1 and $P < 0.05$) with PEDv