

**Title:** Evaluation of the risk of a feed mill being contaminated with PEDv or PDCov -  
**NPB #14-165**

**Investigator:** Laura Greiner

**Institution:** Carthage Innovative Swine Solutions, LLC

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### Industry Summary:

Twenty-four feed mills from various regions (Eastern, Midwest, Western, and Northern Midwest) in the US were evaluated in this study to assess the risk of different areas within a mill to test positive for either porcine epidemic diarrhea virus (PEDv) or swine delta coronavirus (PDCov). Samples were collected at each feed mill for multiple days. Sample areas included: both foot pedals of feed delivery truck, bulk ingredient unloading pit, inside mixer/pellet cooler, mill office floor, inside feed compartment on feed truck, and incoming bagged ingredient truck (inside of truck near site of off-loading). Samples were collected using a technique similar to the Swiffer® technique described by Dee et al. using gauze instead of Swiffers®. Samples were tested for the presence of PEDv and PDCov. Data were analyzed as odds-on-ratios and risk assessments for each area within the mill being tested. Raw percentages demonstrated that no samples tested positive for PEDv. However, 5% of the truck foot pedals and 1% of the bulk ingredient pit tested suspect for PEDv. Porcine delta coronavirus particles were found on 3.4% of the foot pedals of the trucks and 2.2% of the office floors tested suspect. Mills feeding herds that were either PEDv or PDCov positive had a greater probability of having either a suspect or positive test. In addition, for each day a negative test against PEDv or PDCov on the foot pedal was found the chance of a PEDv positive/suspect result declined from 73.6% to 67.7% and PDCov declined from 28% to 17.7%.

Although this study did not determine if positive/suspect samples were in fact capable of producing an infection in an animal, the presence of the virus indicates a risk factor. One mill that was not servicing PDCov positive pigs did have a suspect PDCov PCR, which indicates that herd health status is not the only potential cause for a positive/suspect finding. Furthermore, based on these data, the presence of either PEDv or PDCov particles demonstrates that feed mills and farm owners should review their current biosecurity practices to minimize the potential risk of transferring the virus.

Questions can be directed to: Laura Greiner, [lgreiner@hogvet.com](mailto:lgreiner@hogvet.com), 217-357-2811 (phone)

**Keywords:** Feed mill, PEDv, sampling, risk assessment

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For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • [pork.org](http://pork.org)

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**Scientific Abstract:**

Twenty-four feed mills from various regions (Eastern, Midwest, Western, and Northern Midwest) in the US were evaluated in this study to assess the risk of different areas within a mill to test positive for either porcine epidemic diarrhea virus (PEDv) or swine delta coronavirus (PDCov). Samples were collected at each feed mill for up to 5 days. Sample areas included: both foot pedals of feed delivery truck, bulk ingredient unloading pit, inside mixer/pellet cooler, mill office floor, inside feed compartment on feed truck, and incoming bagged ingredient truck (inside of truck near site of off-loading). Samples were collected using a technique similar to the Swiffer® technique described by Dee et al. In brief, a wet gauze pad was used as the transfer material and was placed into 25 mL of phosphate buffered saline (PBS). The gauze was then squeezed multiple times in the solution and then the supernatant was poured into a sterile, transfer tube. The samples were submitted to diagnostic facilities for PCR testing for PEDv and PDCov. The results of the feed mills for each collection site within the mill and for the mill itself were compiled. Feed mills were assigned a letter for anonymity during analysis and final report. Data were analyzed as probabilities and risk assessments for each area within the mill being tested. In addition, a break-down of the risk of contamination of the mills that already serviced positive herds was evaluated. A summary table was generated from the results above to report the risk of a feed mill being contaminated with either PEDv or PDCov. Raw data percentages demonstrated that no samples tested positive for PEDv. However, 5% of the truck foot pedals and 1% of the bulk ingredient pit tested suspect for PEDv. Porcine delta coronavirus was found on 3.4% of the foot pedals of the trucks and 2.2% of the office floors tested suspect. Of the feed mills tested, 75% of the mills were providing feed to herds infected with PEDv and 21% were feeding herds infected with PDCov. An additional 33% of the feed mills reported “unknown” of the PDCov status of the herds that they were feeding. As the number of days increase, the probability of a positive/suspect result also increases for locations such as the foot pedals, office floor, bulk ingredient pit and the inside of the feed truck compartment. Based on the results by day and the percentage of mills that were feeding positive herds, after one day of negative test results on foot pedals, there is a 74% chance that the foot pedals could test positive for PEDv with additional days of testing. When evaluating the probability of a PDCov positive/suspect result on the foot pedals, there is a 28% chance that the mill will have a positive result in the next four days after the first day of negative results regardless of the health status of the herds being fed from that mill. One feed mill that was servicing PDCov negative herds tested PCR suspect on the office floor, which indicates that the health status of the herds being serviced by the feed mill may not be the only potential cause for a positive/suspect finding. In conclusion, this study demonstrates that both PEDv and PDCov virus particles can be detected at different locations associated with a feed mill. This study does not determine if the virus particles are infectious and therefore, only demonstrates the probably of detecting virus particles at the various locations based on the number of days testing occurs and also based on the health status of the herds being fed from the mill.

**Introduction:** With the outbreak of PEDv and PDCov in the United States, many questions have arisen that have asked the question concerning the movement of the virus throughout the United States. One question that was of interest was the involvement of transport vehicles onto farms. A common transport vehicle that visits multiple farms in a day is the feed truck. Not only is the truck itself of interest, but is the feed mill itself is also of interest. With both feed trucks and staff returning from farms and incoming ingredient trucks delivering feedstuffs, the question is what is the risk of various areas of the mill to be found positive for either virus on any given day. Therefore, the goal of this study was to investigate the risk of key areas in the mill of testing positive for either PEDv or PDCov.

**Objectives:** To quantify the risk of a feed mill testing positive for porcine epidemic diarrhea virus (PEDv) and/or delta corona virus (PDCov).

**Materials & Methods:** Twenty-four feed mills from various regions (Eastern, Midwest, Western, and Northern Midwest) in the US were evaluated in this study. A minimum of six of the feed mills were feeding herds negative for PEDv. For up to 5 days, samples were collected at each feed mill to evaluate the risk of a feed mill testing positive for PEDv and PDCov. Sample areas included: both foot pedals of feed delivery truck, bulk ingredient unloading pit, inside mixer/pellet cooler, mill office floor, inside feed compartment on feed truck, and incoming bagged ingredient truck (inside of truck near site of off-loading).

In brief, sample kits including latex gloves, 50 mL tube with 5 mL of sterile phosphate buffered saline (PBS), Ziploc® bag containing 25 mL of sterile PBS and 4"X4" sterile gauze were prepared before visit to the mill. The sterile gauze was placed in the tube with the PBS so that it was wet and was left in the tube until ready for use.

Before each collection, new latex gloves were worn by the collector. The gauze was removed from the 50 mL tube and wiped over the entire sample area that was roughly one square foot in size. After each collection, the soiled gauze was placed into the Ziploc® bag and squeezed to remove fluid from pad. Fluid was then drained from the bag into its original 50 mL tube and labeled accordingly (Location within mill, Mill ID, Date). The tubes were then placed on ice and in a cooler for transport. Samples were kept frozen in a -20°C freezer for the week until submitted for analysis. Samples were tested for PEDv and PDCov via PCR at either the University of Minnesota Diagnostic Laboratory (St. Paul, MN), Iowa State University Diagnostic Laboratory (Ames, IA), or South Dakota State University (Brookings, SD).

The results of the feed mills for each collection site within the mill and for the mill itself were compiled. Results were identified as positive, suspect, or negative. Suspect findings were based on the individual diagnostic laboratory designated cutoffs. Feed mills were assigned a letter ID for anonymity during analysis and final report. Data were analyzed as probabilities and risk assessments for each area within the mill being tested. In addition, a break-down of the risk of contamination of the mills that already serviced positive herds was evaluated. A summary table was generated from the results above to report the risk of a feed mill testing positive/suspect for either PEDv or PDCov.

## **Results:**

The sampling technique was validated prior to the start of the collection period. In a study conducted by Dr. Jim Lowe (unpublished data), he demonstrated that using gauze pads as the sampling material allowed for a 100% recovery of the sample when the CT value of the original material was <25. In comparison, Swiffer® sampling was less than 100% at a CT value lower 24.

Of the feed mills tested, 75% of the mills were providing feed to herds infected with PEDv and 21% were feeding herds infected with PDCov. An additional 33% of the feed mills reported "unknown" concerning the PDCov status of the herds that they were feeding.

Raw means from the total samples collected per location within the mills for both PEDv and PDCov are presented in Tables 1 and 2, respectively. In short, no samples tested positive for PEDv. However, 5% of the truck foot pedals and 1% of the bulk ingredient pit tested suspect for PEDv. Porcine delta coronavirus was found on 3.4% of the foot pedals of the trucks and 2.2% of the office floors tested suspect. One mill that was currently not feeding PDCov positive herds did have a PDCov suspect result on the office floor.

Tables 3 and 4 demonstrate the probability of negative results based on the health status of the herds in which the mill was feeding.

Tables 5 and 6 further break down the probability of either PEDv or PDCov positive/suspect results at the various tested locations within the mill based on the health status of the pigs being fed from the mill. As the number of days increase, the probability of a positive/suspect result also increases for

locations such as the foot pedals, office floor, bulk ingredient pit and the inside of the feed truck compartment. In short, since no mills tested positive for PEDv but a few did have suspect results, the likelihood of the presence of virus particles at the mills feeding PEDv positive herds after consecutive days of testing is lower compared to mills that are feeding PDCov positive herds.

However, as denoted above, the percent of feed mills feeding PEDv positive herds was higher than those feeding known PDCov positive herds. Therefore, Tables 7 and 8 and also in Graphs 1 and 2, demonstrate the probabilities of a mill testing positive based on the number of days it has tested negative with percentage of mills feeding positive herds included in the analysis. For example, after one day of negative test results on foot pedals, there is a 74% chance that the foot pedals could test positive for PEDv with additional days of testing. When evaluating the probability of finding a PDCov positive result on the foot pedals, there is a 28% chance that the mill will find a positive result in the next four days after the first day of negative results regardless of the health status of the herds being fed from the mill.

### **Discussion:**

Testing for PEDv resulted in a number of PCR suspect results on the foot pedals of feed delivery trucks and mill office floors although no samples tested positive for virus. However, PDCov PCR positive samples were found on feed truck foot pedals and several PCR suspect results were found on the office floor of the tested feed mills. The tables presented also demonstrate the probability of various locations within the mill testing positive based on the health status of the pigs and also based on how many days of testing have been conducted. Mills currently feeding PEDv or PDCov positive pigs had a higher chance of having a positive/suspect PCR test. One mill that was currently not feeding PDCov positive herds did have a PDCov suspect result on the office floor indicating that the health status of the herds being serviced by the mill are not the only potential sources that could lead to a positive/suspect PCR.

Although this study did not determine if the suspect or positive PCR samples would result in creating a clinical disease in an animal, the fact that samples were found to be positive indicate that virus particles were present in the feed mill or the delivery trucks. Based on the findings, key areas within the mill need to be monitored to reduce the chance of detecting either virus, and biosecurity protocols will need continued evaluation to minimize the risk.

Table 1. Raw percentages of the findings for PEDv testing

		Office floor	Mixer / cooler	Bulk ingredient pit	Ingredient delivery trucks	Feed transport truck foot pedals	Inside feed truck compartment
Samples							
	Total	100	99	100	74	101	100
PCR results							
	Positive, %	0.0	0.0	0.0	0.0	0.0	0.0
	Suspect, %	0.0	0.0	1.0	0.0	5.0	1.0
	Negative, %	100.0	100.0	99.0	100.0	95.0	99.0

Table 2. Raw percentages of the findings for Delta coronavirus testing

		Office floor	Mixer / cooler	Bulk ingredient pit	Ingredient delivery trucks	Feed transport truck foot pedals	Inside feed truck compartment
Samples							
	Total	100	99	100	74	101	100
PCR results							
	Positive, %	0.0	0.0	0.0	0.0	3.4	0.0
	Suspect, %	2.2	0.0	0.0	0.0	1.1	0.0
	Negative, %	97.8	100.0	100.0	100.0	95.5	100.0

Table 3. Average probability of negative PEDV results based on location and the PEDv status (PEDv+ (positive) or PEDv- (negative) of the herds being fed from the mill.

Average (over 5 samples) Probability of Negative Test Results							
		Office Floor	Mixer	Foot Pedal	Bulk Ingred Pit	Ingredient Truck	Feed Compartment
Mills feeding PEDv+ pigs	Test ~+	1.000	1.000	0.931	0.983	1.000	0.986
	Test-	1.000	1.000	0.931	0.983	1.000	0.986
Mills feeding PEDv- pigs	Test ~+	1.000	1.000	1.000	1.000	1.000	1.000
	Test-	1.000	1.000	1.000	1.000	1.000	1.000
Total mills	Test ~+	1.000	1.000	0.951	0.989	1.000	0.990
	Test-	1.000	1.000	0.951	0.989	1.000	0.990

Test ~+ : Either a positive or suspect result

Test - : Negative results

Table 4. Average probability of negative PDCov results based on location and the PDCoV status (PDCoV+ (positive), PDCoV? (unknown), PDCov- (negative)) of the herds being fed from the mill.

Average (over 5 samples) Probability of Negative Test Results							
		Office Floor	Mixer	Foot Pedal	Bulk Ingred Pit	Ingredient Truck	Feed Compartment
Mills feeding PDCov+ pigs	Test+	1.000	1.000	0.840	1.000	1.000	1.000
	Test-	1.000	1.000	0.840	1.000	1.000	1.000
Mills Feeding PDCov? pigs	Test+	0.967	1.000	1.000	1.000	1.000	1.000
	Test-	0.967	1.000	1.000	1.000	1.000	1.000
Mills feeding PDCov- pigs	Test+	0.967	1.000	1.000	1.000	1.000	1.000
	Test-	0.967	1.000	1.000	1.000	1.000	1.000
Total mills	Test+	0.982	1.000	0.923	1.000	1.000	1.000
	Test-	0.982	1.000	0.923	1.000	1.000	1.000

Table 5. Probability of having a PEDv positive/suspect result based on the number of days tested and the PEDv status (PEDv+ (positive) or PEDv- (negative) of the herds being fed from the mill.

Probability of Any Positive Test Results by Sample Days							
		Office Floor	Mixer	Truck Pedal	Bulk Pit	Ingredient Truck	Truck Compartment
Mills feeding PEDv+ pigs		Sample Days					
Test ~+	1	0.000	0.000	0.069	0.017	0.000	0.014
	2	0.000	0.000	0.133	0.033	0.000	0.028
	3	0.000	0.000	0.193	0.049	0.000	0.042
	4	0.000	0.000	0.248	0.065	0.000	0.056
	5	0.000	0.000	0.300	0.081	0.000	0.069
Mills feeding PEDv- pigs		Sample Days					
Test ~+	1	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000

Test ~+ : Either a positive or suspect result

Test - : Negative results



Table 6. Probability of having a PDCov positive/suspect result based on the number of days tested and the PDCoV status (PDCoV+ (positive), PDCoV? (unknown), PDCov- (negative)) of the herds being fed from the mill.

Probability of Any Positive/Suspicious Test Results by Sample Days							
		Office Floor	Mixer	Foot Pedal	Bulk Pit	Ingredient Truck	Feed Compartment
Mills feeding PDCov pigs							
	Sample Days						
Test+	1	0.000	0.000	0.160	0.000	0.000	0.000
	2	0.000	0.000	0.294	0.000	0.000	0.000
	3	0.000	0.000	0.407	0.000	0.000	0.000
	4	0.000	0.000	0.502	0.000	0.000	0.000
	5	0.000	0.000	0.582	0.000	0.000	0.000
Mills feeding PDCov? pigs							
	Sample Days						
Test+	1	0.033	0.000	0.000	0.000	0.000	0.000
	2	0.066	0.000	0.000	0.000	0.000	0.000
	3	0.097	0.000	0.000	0.000	0.000	0.000
	4	0.127	0.000	0.000	0.000	0.000	0.000
	5	0.156	0.000	0.000	0.000	0.000	0.000
Mills feeding PDCov- pigs							
	Sample Days						
Test+	1	0.033	0.000	0.000	0.000	0.000	0.000
	2	0.066	0.000	0.000	0.000	0.000	0.000
	3	0.097	0.000	0.000	0.000	0.000	0.000
	4	0.127	0.000	0.000	0.000	0.000	0.000
	5	0.156	0.000	0.000	0.000	0.000	0.000

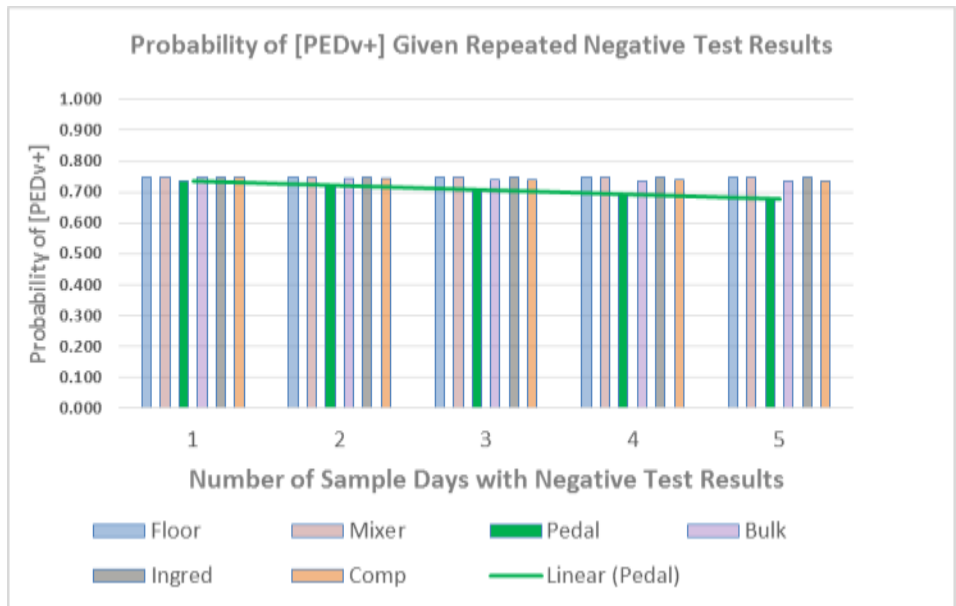
Table 7. Probability of a mill have a PEDv positive test result based on the number of days of receiving negative test results.

Probability of PEDv+ in Mill Given Negative Test Results by Sample Days						
Sample Days	Office Floor	Mixer	Foot Pedal	Bulk Pit	Ingredient Truck	Truck Compartment
1	0.750	0.750	0.736	0.747	0.750	0.747
2	0.750	0.750	0.722	0.744	0.750	0.745
3	0.750	0.750	0.708	0.740	0.750	0.742
4	0.750	0.750	0.693	0.737	0.750	0.739
5	0.750	0.750	0.677	0.734	0.750	0.736

Table 8. Probability of a mill having a PDCov positive test result based on the number of days of receiving negative test results.

Probability of PDCov+ in Mill Given Negative Test Results by Sample Days						
Sample Days	Office Floor	Mixer	Truck Pedal	Bulk Pit	Ingredient Truck	Truck Compartment
1	0.313	0.313	0.281	0.313	0.313	0.313
2	0.313	0.313	0.252	0.313	0.313	0.313
3	0.313	0.313	0.224	0.313	0.313	0.313
4	0.313	0.313	0.199	0.313	0.313	0.313
5	0.313	0.313	0.176	0.313	0.313	0.313

Graph 1. Graph of probability of having PEDv + results based on the number of sequential negative results.



Graph 2. Graph of probability of having PDCov + results based on the number of sequential negative results.

