

**Title:** Quantifying the presence of viral material in feed delivered in Iowa – #20-104 IPPA

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### Industry Summary:

Despite evaluating a feed sample for 14 feed mills for 16 weeks, there was no detection of PRRSV, PEDV, PDCoV, and TGEV in any of the 224 collected feed samples. These results do not disprove that feed can be a fomite of domestic and foreign animal diseases. However, these findings are the first known evaluation of infected feed prevalence. The goal of this project was to provide a data point for pork producers to evaluate the actual risk of feed being a fomite of PRRSV, PEDV, PDCoV, and TGEV and what the return over investment scenarios are for using the various feed mitigants and sanitation products available today.

### Key Findings:

- There was no observed viral contamination (PRRSV, PEDV, PDCoV, and TGEV) of feed delivered in Iowa in these 224 feed samples
  - Collected once per week across 14 Iowa based feed mills over 16 weeks
- In this data set, the risk of viral material in feed for commercial swine consumption was at least less than 1 in 224

**Keywords:** biosecurity, feed, PEDV, swine, viral

### Scientific Abstract:

Experimental models have shown that viral genetic material can be found in feedstuffs for over 150 days and experimental viral contamination of feed can cause viral diseases in pigs. However, the actual risk of feed being a fomite for viruses that cause PRRS, PED, PDCoV, and TGE in everyday pork production has yet to be determined. Thus, our objective was to quantify the viral presence and load of PRRSV, PEDV, PDCoV, and TGEV in 224 feed samples delivered throughout Iowa in two periods of the year. The first period was from November 29<sup>th</sup>, 2020 through February 20<sup>th</sup>, 2021, during the period of suspected seasonally elevated virus pressure.

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The second period was from June 6th through July 3rd, 2021, during the period of suspected decreased virus pressure. We hypothesized that fewer than 2 of the 224 feed samples would result in a positive PCR test. To test our hypothesis and accomplish our experimental objective, 1 feed sample was collected per feed mill (14) for each of 16 weeks (12 in the winter collection period and four in the summer collection period; [n = 224]). Each feed sample was collected at the mill during the loading of the feed truck before delivery. Post collection each feed sample was identified and then shipped to the Veterinary Diagnostic Laboratory at Iowa State University. All collected feed samples were tested via PRRSV PCR and PEDV/PDCoV/TGEV multiplex PCR. The Veterinary Diagnostic Laboratory at Iowa State University declared Ct values >37 a negative (-) result for PRRSV and >36 a negative (-) result for PEDV, PDCoV, and TGEV. Of the 224 collected feed samples, 0 tested positive for PRRSV. Of the 224 collected feed samples 0, tested positive for PEDV, PDCoV, and TGEV. These results do not disprove that feed can be a fomite of domestic and foreign animal diseases. These results just provide a data point for pork producers to evaluate the actual risk of feed being a fomite of PRRSV, PEDV, PDCoV, and TGEV and what the return over investment scenarios are for using the various feed mitigants and sanitation products available today.

### **Introduction:**

Iowa pork producers are being bombarded by ingredient suppliers selling a multitude of feed products and strategies designed to mitigate a variety of viruses that cause disease in pigs and thereby reduce production. However, the actual risk of feed being a fomite for viruses that cause Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Epidemic Diarrhea Virus (PEDV), Porcine Deltacoronavirus (PDCoV), and Transmissible Gastroenteritis Virus (TGEV) in everyday pork production has yet to be determined. It is generally accepted that viral genetic material can be found in feedstuffs for over 150 days during controlled *in vitro* experiments (Dee et al., 2019). Furthermore, experimental viral contamination of feed can cause viral diseases in pigs (Dee et al., 2019). However, characterization and quantification of viral genetic material presence in feed being delivered daily in Iowa has not been undertaken. This information is necessary for pork producers to truly assess the need for feed viral mitigation products and strategies.

### **Objectives:**

Our objective is to characterize and quantify the presence of PRRSV, PEDV, PDCoV, and TGEV in 224 feed samples delivered throughout Iowa during two periods of the year: November 29th, 2020 through February 20th, 2021 (elevated virus pressure seasonal period of the year) and June 6th through July 3rd, 2021 (decreased virus pressure seasonal period of the year).

### **Materials and Methods:**

This experiment was conducted by collecting 224 feed samples from 14 different feed mills located across northwest, southwest, northeast, and southeast Iowa (Figure 1). Each feed mill was sampled once per week for 16 weeks (168 samples) from November 29<sup>th</sup>, 2020 through February 20<sup>th</sup>, 2021. Each mill was sampled again once per week for 4 weeks from June 6<sup>th</sup>, 2021 through July 3<sup>rd</sup>, 2021 (56 samples). These 2 different sampling intervals were selected to represent suspected seasonal differences in risk of viral contamination of feed (Autumn/Winter = elevated risk, Summer = reduced risk).

Of these 14 feed mills (Table 1): 1 feed mill applied Sal CURB (Kemin Industries, Des Moines, Iowa) for feed viral mitigation purposes during the winter feed sample collection period only, one feed mill produced only starter pellets heated to ~160°F, 3 feed mills produced finishing pellets heated to ~180°F (these 3 feed mills could send in either a mash or pelleted form feed sample). All other feed samples collected were of meal form and were not treated with any feed mitigant (Table 1). Furthermore, none of the feed mills in this experiment allowed porcine plasma or meat and bone meal on their premises (Table 1). All imported ingredients used in any premix or feed were held in isolation for 60 days before manufacturing. All premixes used in this experiment tested negative for PEDV/PDCoV/TGEV before being shipped to the feed mill where final feed manufacturing occurred.

All feed samples were collected when the finished feed was loaded onto the feed truck before on-site delivery to avoid any viral contamination from the pigs currently housed. All sample preparation and molecular analysis were conducted at the Iowa State University Veterinary Diagnostic Laboratory. All collected feed samples were individually tested via PRRSV PCR and PEDV/PDCoV/TGEV multiplex PCR.

### **Results:**

The Iowa State Veterinary Diagnostic Laboratory uses a cutoff value of >37 for PRRSV PCR and >36 for the PEDV/PDCoV/TGEV multiplex PCR to characterize a result as negative. Using these cutoffs, none of the 224 collected feed samples tested positive for PRRSV (Table 2), PEDV, PDCoV, or TGEV (Table 3).

### **Discussion:**

For a feed to be a fomite of virus transmission in commercial pig production, the feed or an ingredient in the feed must be contaminated by a virus, the virus must survive the transportation and manufacturing process, and must be consumed by the pig in enough quantity to cause disease. Experimental models have shown that viral genetic material can be found in feedstuffs for over 150 days and experimental viral contamination of feed can cause viral diseases in pigs (Dee et al., 2019). Furthermore, during the initial North American PEDV outbreak, there were several case studies where feed was identified as a fomite (Dee et al., 2020). However, in each of those published case studies the feed that had a positive PCR result was collected after the disease confirmation of the pigs. Our research objective was to characterize quantify the presence of PRRSV, PEDV, PDCoV, and TGEV in finished feed samples delivered in Iowa before the arrival at the barn. Despite testing 14 feed mills for 16 weeks, there was no detection of genetic material of PRRSV, PEDV, PDCoV, and TGEV in any of the 224 samples tested. These results do not disprove that feed can be a fomite of domestic and foreign animal diseases. These results just provide a data point for pork producers to evaluate the actual risk of feed being a fomite of PRRSV, PEDV, PDCoV, and TGEV and what the return over investment scenarios are for using the various feed mitigants and sanitation products available today.

**Table 1.** Description of feed samples collected

Feed mill	Iowa location	Sal CURB <sup>1</sup> application	Pelleted feed	Meal feed	Use of porcine plasma or meat and bone meal	Parameter
						Premix tested negative for PEDV/PDCoV/TGEV prior to arrival at mill
A	Northwest	No	Yes <sup>2</sup>	No	No	Yes
B	Northwest	No	Yes <sup>3</sup>	Yes	No	Yes
C	Northwest	No	Yes <sup>3</sup>	Yes	No	Yes
D	Northwest	No	No	Yes	No	Yes
E	Northwest	No	No	Yes	No	Yes
F	Northwest	No	No	Yes	No	Yes
G	Northwest	No	No	Yes	No	Yes
H	Northwest	No	No	Yes	No	Yes
I	Northwest	No	No	Yes	No	Yes
J	Southwest	Yes	No	Yes	No	Yes
K	Southwest	No	No	Yes	No	Yes
L	Northeast	No	Yes <sup>3</sup>	Yes	No	Yes
M	Northeast	No	No	Yes	No	Yes
N	Southeast	No	No	Yes	No	Yes

<sup>1</sup>Kemin Industries, Des Moines, IA.

<sup>2</sup>Starter pellet (heated to ~160°F).

<sup>3</sup>Finishing pellet (heated to ~180°F).

**Table 2.** PCR Ct results on quantifying the mRNA presence of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in collected feed samples from 14 Iowa feed mills over 16 weeks<sup>1</sup>

Feed mill	Collection week <sup>2</sup>															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
B	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
C	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
D	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
E	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
F	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
G	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
H	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
I	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
J	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
K	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
L	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
M	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-
N	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-	>37/-

<sup>1</sup>Veterinary Diagnostic Laboratory at Iowa State University declared Ct values >37 a negative (-) result.

<sup>2</sup>Collection week 1 through 12 was November 29th, 2020 through February 20th, 2021 [elevated virus pressure seasonal period of year] and collection week 13 through 16 was June 6th through July 3rd, 2021 [decreased virus pressure seasonal period of year]).

**Table 3.** PCR Ct results on quantifying the mRNA presence of Porcine Epidemic Diarrhea Virus (PEDV), Porcine Deltacoronavirus (PDCoV), and Transmissible Gastroenteritis Virus (TGEV) in collected feed samples from 14 Iowa feed mills over 16 weeks<sup>1</sup>

Feed mill	Collection week <sup>2</sup>															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
B	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
C	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
D	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
E	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
F	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
G	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
H	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
I	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
J	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
K	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
L	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
M	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-
N	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-	>36/-

<sup>1</sup>Veterinary Diagnostic Laboratory at Iowa State University declared Ct values >36 a negative (-) result.

<sup>2</sup>Collection week 1 through 12 was November 29th, 2020 through February 20th, 2021 [elevated virus pressure seasonal period of year] and collection week 13 through 16 was June 6th through July 3rd, 2021 [decreased virus pressure seasonal period of year].

**Figure 1.** Location and number of feed mills sampled throughout each Iowa region during this experiment

