SWINE HEALTH INFORMATION CENTER
FINAL RESEARCH GRANT REPORT FORMAT

Project Title and Project identification number:

Evaluating methods of extraction for pathogen detection in feed ingredients and environmental samples from feed mills – SHIC #21-065

Principal Investigator: Diego G. Diel

Institution: Cornell University

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Industry Summary: Feed biosecurity has been an area of significant interest to the swine industry. Early studies with porcine epidemic diarrhea virus (PEDV) suggested that the virus may have been transmitted through feed. Recent experimental evidence confirmed that PEDV, SVA and FMDV can indeed be transmitted through contaminated feed that is ingested naturally by susceptible pigs. One way of reducing the risk of pathogen transmission through feed is to test feed ingredients and feed before they are introduced into farms and fed to pigs. This would only be possible if sampling and nucleic acid extraction methods would allow efficient detection of pathogens in feed. In this study we focused on comparing the performance of three commercially available nucleic acid extraction kits (CORE, IndiMag, MVP II). These kits were tested in samples that were spiked with PRRSV, SVA and PEDV and that were previously collected as part of a transportation study and tested in another VDL. Our results show that the Core extraction kit outperformed the other two kits evaluated in the present study and previously used in another VDL that originally had tested the samples. Overall samples extracted with the Core kit presented lower Ct value (at least for PRRSV and SVA) and a higher sensitivity when compared to samples extracted with MVPII or the IndiMag, One of the key issues that remains to be addressed in future studies is the sampling method to be used for large volumes of feed or feed ingredients.

Keywords: feed, biosecurity, nucleic acid extraction, PRRSV, SVA, PEDV

Scientific Abstract: Feed biosecurity has been an area of significant interest to the swine industry. Early studies with porcine epidemic diarrhea virus (PEDV) suggested that the virus may have been transmitted through feed. Recent experimental evidence confirmed that PEDV, SVA and FMDV can indeed be transmitted through contaminated feed that is ingested naturally by susceptible pigs. One way of reducing the risk of pathogen transmission through feed is to test feed ingredients and feed before they are introduced into farms and fed to pigs. This would only be possible if sampling and nucleic acid extraction methods would allow efficient detection of pathogens in feed. In this study we focused on comparing the performance of three commercially available nucleic acid extraction kits (CORE, IndiMag, MVP II). These kits were tested in samples that were spiked with PRRSV, SVA and PEDV and that were previously collected as part of a transportation study and tested in another VDL. Our results show that the Core extraction kit outperformed the other two kits evaluated in the present study and previously used in another VDL that originally had tested the samples. Overall samples extracted with the Core kit presented lower Ct value (at least for PRRSV and SVA) and a higher sensitivity when compared to samples extracted with MVPII or the IndiMag, One of the key issues that remains to be addressed in future studies is the sampling method to be used for large volumes of feed or feed ingredients.

Introduction:

The introduction of Porcine Epidemic Diarrhea Virus (PEDV) into the US in 2013 caused significant economic losses to the US swine industry [1]. The introduction of the virus into the US resulted in the loss of approximately 7 million pigs or 10% of the annual pig population [1]. The root cause of PEDV introduction to the US has not been conclusively determined; however, contaminated feed and feed ingredients may have served as vehicles for PEDV introduction, as PEDV transmission though contaminated feed has been well documented [2]. Furthermore, given the fact that the original PEDV strain detected in the US shared 99.7-99.8% nucleotide identity with a Chinese PEDV strain [3], actively circulating in China raised the question of whether contaminated feed could have served as a vehicle for the initial virus entry into the US swine population. By using a Trans-Pacific transportation model, Dee and collaborators evaluated the possibility of PEDV surviving a trip from China to the US [4]. By spiking feed ingredients commonly imported from China to the US with PEDV, and subjecting the mixtures to environmental conditions simulating a 37-day trip from Beijing to Des Moines, IA [5], the authors showed that PEDV survived the transport period in five key ingredients used to formulate porcine rations, including soybean meal (organic and conventional), vitamin D, lysine hydrochloride and choline chloride [5].

These results raised important questions as to whether contaminated animal feed and feed ingredients could serve as vehicles for the spread of other viral diseases between countries. A large scale study conducted by our group in collaboration with Dr. Scott Dee revealed that indeed several important swine pathogens, including Senecavirus A (SVA) and African Swine Fever Virus (ASFV) survive in feed ingredients under environmental conditions mimicking transportation of the target ingredients from Asia and/or Europe into the US [6]. In fact, these two viruses survived in a higher number of feed ingredients than almost all other viruses we tested, demonstrating that ASFV and SVA are extremely stable in feed and are appropriate targets for further research. Most importantly, subsequent studies confirmed that significant pathogens of swine including SVA, FMDV and ASFV [7–9] can be transmitted to pigs following natural consumption of contaminated feed. These findings highlight the importance of feed biosecurity and underscore the need for nucleic acid extraction protocols for pathogen detection in feed ingredients.

The present project is an extension of our NPB project #19-170 “Validating pathogen nucleic acid extraction from animal feed and feed ingredients”. The goal of the present project was to evaluate the extraction methods developed in the NPB proposal in samples collected in field studies conducted by Dr. Scott Dee and Cassie Jones. The two studies collected feed samples (conventional soybean meal, organic soybean meal and complete finishing feed) or environmental samples from feed totes that have been spiked with Porcine Reproductive and Respiratory Virus (PRRSV), Senecavirus A (SVA) or Porcine Epidemic Diarrhea virus (PEDV) (Dee study) or from. We evaluated the extraction methods using the MagMax Core extraction kit and compared its performance with Magmax Viral Pathogen II (MVP II) and the Indimag Pathogen kit.

Objectives: The goal of the present project was to compare nucleic acid extraction methods for pathogen nucleic acid extraction from feed and feed ingredients.

Materials & Methods:

Samples included in this project. The samples tested in the present study were kindly provided by Dr. Scott Dee (Pipestone Applied Research). These samples were collected during a transportation study conducted by Dr. Dee to assess viability of SVA, PRRSV and PEDV in feed ingredients following a continental trip across the United States. It is important to note that the samples were originally tested in another AAVLD accredited Veterinary Diagnostic Laboratory in the US but were provided and tested blinded in the present study. Original testing results were only provided by Dr. Dee after the samples had been processed and tested at the Cornell Animal

Health Diagnostic Center (AHDC). A brief description of the experimental design and sampling method used in the transportation study by Dr. Dee is provided below for reference.

Transportation study experimental design. The experimental design described below is part of Dr. Scott Dee's transportation study and is provided here to define the samples and sampling methods that were used to obtain the feed samples that will be tested with the different extraction methods compared in the present study.

Viruses and feed ingredients: Viruses included in this study include PRRSV-174, PEDV, and SVA. Ingredients spiked with these viruses consisted of 1-ton totes of conventional soybean meal (2 totes), 1-ton totes of organic soybean meal (2 totes) and 1-ton totes of complete finishing feed (2 inoculated totes and 1 uninoculated control tote). Brand new totes (polyethylene) were used to hold each ton of feed.

Organization of totes: Each tote had dimensions of 48 inches in length, 48 inches in width, and 54 inches in height. Totes were stored in the trailer of a commercial transport vehicle for inoculation and sampling. Based on the Association of American Feed Control Officials Feed Inspection Manual, each tote was sampled in 10 sampling areas (#1-10), using a "double X" pattern, an approach that has recently been validated for the detection of PEDV in bulk feed by Jones and collaborators.

Inoculation of totes: Each tote was inoculated with 10 mL of viral inoculum frozen at -80^{0}C into a solid cube of ice. Each cube contained 1 x 10^5 $TCID_{50}$ of PRRSV 174, PEDV, and SVA. Ice cubes containing the target viruses were randomly dropped into the feed in the tote halfway through the filling process to simulate a random hotspot point of contamination.

Controls: To serve as a negative control, an additional 1-ton tote of conventional soybean meal will be placed into the transport vehicle. It will be inoculated as described; however, an ice cube of sterile saline will be used. For the purposes of positive controls, two 30 g samples of each ingredient will be placed into individual sterile 50 mL plastic centrifuge tubes with vented caps. To represent the volume of liquid from the ice cube (10 mL) used in the 1-ton totes, these 30 g controls will be inoculated with 0.3 ml of viral mixture. These samples will be stored in a box placed next to the totes in the trailer.

Sampling: Prior to initiation of the road trip and upon completion of the trip, 10 areas in each tote were sampled using a grain probe (Seedburo Equipment Company) as described by Jones and others. The 10 samples from each tote were mixed into one tote-specific composite sample for testing. The grain probe was sanitized between totes per Jones, using an air blower for dust removal, followed by 70% ethanol spray. The probe was wiped, and the spray allowed to evaporate prior to re-using. A total of 6 virus spiked samples (2, C-SBM, 2, O-SBM, and 2 complete feed) plus 1 negative control (C-SBM) were collected prior to initiation and at the completion of the trip, for a total of 14 feed samples.

Sample processing, nucleic acid extraction and testing. We received two sets of samples from Dr. Dee's transportation study. The first set of samples (n = 28) consisted of the supernatants from the same feed totes that had been previously processed and tested in another AAVLD accredited laboratory, while the second set (n = 14) consisted of actual feed or feed ingredients (~1 lb, C-SBM, O-SBM, Complete feed) collected during the transportation study from the spiked feed totes.

The first set of samples (n = 28) consisted of samples collected in the same transportation experiment in duplicate that had been previously processed and tested in another VDL. From

these samples we only received the supernatant after the feed matrix had already been reconstituted in liquid for nucleic acid extraction. These samples were processed as bulk feed samples in paint gallons, in which 1L of PBS was used to reconstitute 1 lb of each feed ingredient. These samples were only subjected to nucleic acid extraction and RT-PCR at Cornell AHDC. All nucleic acid extractions with the Core and MVP II kits were performed in the KingFisher Flex or IndiMag instruments following manufacturer's protocols.

The 1-lb feed samples that were received were processed as follows. Three 5 g samples (three replicates) of the composite 1-lb feed ingredient were collected and placed in a sterile 50 ml conical tube. Next, 15 ml of sterile PBS were added to each of the tubes containing the 5 g of feed and the tubes were subjected to vortexing for 10-15 s. After vortexing all samples were centrifuged at 10,000 x g for 10 minutes. The supernatant was harvested and transferred to sterile 2 ml tubes and stored at -80 for further processing.

The transportation samples described above were tested for the presence of PRRSV, SVA and PEDV viral RNA at the Cornell Animal Health Diagnostic Center. Before real-time PCR testing (using commercially available PCR assays – Tetracore Inc.) nucleic acid was extracted from the samples using the new extraction method optimized in our lab (MagMax Core extraction kit) and the traditional extraction method used in our previous studies of pathogen detection in feed (MagMax Pathogen II kit) and with a third extraction method (IndiMag Pathogen kit).

Results and Discussion

In this study we evaluated three nucleic acid extraction protocols for use in feed ingredients: MagMax CORE, IndiMag, and MVPII. These extraction protocols were performed in samples collected from feed totes containing 1 ton of each feed ingredient. The initial comparison performed in our study involved a direct comparison between three extraction methods performed at Cornell AHDC (MagMax Core, IndiMag and MVP II). This study was conducted with the 28 sample supernatants received from another VDL that had previously processed and tested the samples for PRRSV, SVA and PEDV. A summary of the results obtained in this study is presented in **Table 1**.

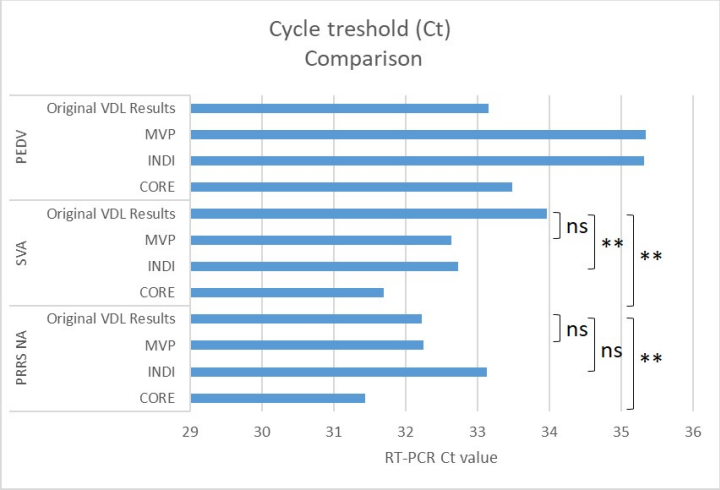
Table 1 – Comparison of extraction methods and RT-PCR results for pathogen detection in feed.

	PRRS NA				SVA				PEDV			
Sample ID	CORE	INDI	MVP	Original VDL Results	CORE	INDI	MVP	Original VDL Results	CORE	INDI	MVP	Original VDL Results
1	30.5104	34.0119	36.1803	33.51	30.9678	33.5602	35.2987	34.7827	34.2208	36.3441	39.1154	37.78
2	29.5368	31.4061	ND	32.63	30.0088	31.2616	ND	33.3605	33.0355	33.796	ND	37.18
3	31.5605	32.4847	32.031	34.51	30.7801	32.2019	30.9399	34.5992	33.226	35.5278	35.6886	35.11
4	31.084	33.1125	31.5925	34.08	34.1309	36.4393	35.0916	36.1911	32.8499	39.5359	34.1054	35.22
5	38.7804	ND	ND	ND	32.487	33.6559	31.6779	36.0372	39.6849	ND	ND	ND
6	ND	ND	ND	ND	31.2789	31.8301	30.9189	35.1156	38.1435	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	33.1719	33.8209	32.3939	35.5835	ND	ND	ND	ND
9	32.4058	38.0679	40.6338	34.4	32.27	35.5056	35.0033	35.682	35.2825	ND	43.2811	ND
10	39.4357	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	31.5177	35.2895	33.5428	34.12	33.714	34.0387	33.334	35.348	34.2079	37.3658	34.8082	37.6
12	ND	ND	ND	ND	35.8868	36.687	34.8207	ND	ND	ND	ND	ND
13	38.4474	ND	ND	ND	32.4426	32.2799	31.2065	35.5254	ND	ND	38.7314	ND
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	27.8928	28.3956	27.9845	30.65	28.6801	29.0889	29.0672	31.45	29.3273	30.0068	29.5414	30.65
16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	27.5207	32.5719	28.0662	31.75	29.512	31.7721	30.9747	32.83	30.8947	36.3209	32.9265	31.75
18	25.3997	30.1987	25.4675	28.92	27.2428	29.5869	28.0049	29.63	26.7273	31.8918	28.8187	28.92
19	27.3504	33.1637	28.0695	30.4	30.2008	30.2555	29.4622	32.21	30.8497	32.5401	31.6291	30.4
20	39.6483	ND	ND	ND	35.9996	ND	ND	ND	ND	ND	ND	ND
21	27.9438	32.6269	33.6718	31.06	29.2163	31.6425	37.3451	32.61	34.9264	ND	37.8831	31.06
22	27.9012	29.9124	28.3936	30.75	31.0606	32.3156	30.7372	33.14	32.9748	34.5704	34.4365	30.75
23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	27.9028	31.7745	28.4353	30.67	30.5106	32.4566	31.0246	32.52	31.625	36.5892	33.529	30.67
25	28.806	32.4741	38.0721	31.21	29.7246	32.2384	36.1237	31.88	34.9631	35.6721	ND	ND
26	28.9524	32.5477	35.3355	31.13	30.1539	31.7931	35.1073	32.7	33.8584	35.6235	35.5455	31.21
27	35.4065	39.3592	35.3821	ND	37.1753	ND	ND	37.02	37.1536	ND	38.9176	ND
28	32.1025	35.91	33.1191	35.81	32.3761	35.0735	34.3018	35.11	32.2989	38.6441	36.465	35.81

ND: not detected.

The average CT value obtained in RT-PCR for PRRSV, SVA and PEDV after samples were extracted with the CORE, INDI, MVP or the original extraction at the testing VDL were compared. As shown in **Fig. 1** extraction with the MagMax CORE extraction kit resulted in lower average Ct values when samples were tested with the Tetracore PRRSV and SVA assays when compared to the original VDL results obtained with the same PCR assays (**, p>0.005).

Figure 1. RT-PCR cycle threshold comparison following nucleic acid extraction with MagMax Core, IndiMag Pathogen, MagMax Viral Pathogen II (MVP) kit. Ns, no significance, **, p>0.005.



We have also assessed the diagnostic sensitivity of the different extraction methods compared in our study using the sample set that was provided to us by Dr. Dee. From the 28 samples we received from Dr. Dee, 24 were collected from feed totes that were spiked with PRRSV, SVA and PEDV as described in the material and methods section of this report. In **Table 2A** bellow, we provide a summary of the number expected results for each target virus and the actual number of positive and negative samples detected in the original VDL that tested these samples and after the samples were extracted with MagMax Core, IndiMag or the MVP kit. It is important to note that all 4 negative control samples tested negative in the original VDL and at Cornell AHDC. The number of positive samples detected after each extraction method, although, varied between the methods used (**Table 2A**).

These numbers were used to estimate the sensitivity and specificity of the extraction methods used. As shown in **Table 2B**, extraction with the MagMax CORE kit resulted in higher sensitivity for all target viruses (87.5% for PRRSV, 95.83% for SVA and 79.17% for PEDV) when compared to the other methods used at AHDC (IndiMag and MVP) and to the method used in the VDL that originally tested these samples.

Table 2. Summary of detection and sensitivity of RT-PCRs for PRRSV, SVA and PEDV following nucleic acid extraction with three different protocols.

A

		PRRS NA				SVA				PEDV			
	Expected	**CORE**	**INDI**	**MVP**	**Original VDL Results**	**CORE**	INDI	MVP	**Original VDL Results**	**CORE**	INDI	MVP	**Original VDL Results**
Pos	24	21	17	16	16	23	21	20	21	19	14	16	14
Neg	4	7	11	12	12	5	7	8	7	9	14	12	14

B

2x2

	CORE			INDI			MVP			VDL Results	
PRRSV	21	0	21	17	0	17	16	0	17	16	0
	3	4	7	7	4	11	8	4	11	8	4
	24	4		24	4		24	4		24	4
Se	87.5			70.8			66.67			66.67	
Sp	100			100			100			100	

	CORE			INDI			MVP			VDL Results	
SVA	23	0	21	21	0	17	20	0	17	21	0
	1	4	7	3	4	11	4	4	11	3	4
	24	4		24	4		24	4		24	4
Se	95.83			87.5			83.33			87.50	
Sp	100			100			100			100	

	CORE			INDI			MVP			VDL Results	
SVA	19	0	21	17	0	17	16	0	17	14	0
	5	4	7	7	4	11	8	4	11	10	4
	24	4		24	4		24	4		24	4
Se	79.17			70.8			66.67			58.33	
Sp	100			100			100			100	

We also evaluated the efficiency of extractions in small size samples (5 g samples) that were obtained from the first 14 samples (Sample 1-14) presented in **Table 1**. Our laboratory received 1 lb of each feed ingredient 1-14 above and we took three independent 5 g samples from these 14 ingredients (replicate 1, 2 and 3 shown in **Table 3**). Each of these 5 g samples was processed as described in the material and methods and the supernatant subjected to nucleic acid using the MagMax Core and IndiMag kits, which were the two kits with the best performance in the large batch samples (**Table 1** and **2** above). All samples were tested using the Tetracore PRRSV, SVA and PEDV RT-PCR kits. Results of these studies are presented in **Table 3**.

Table 3 – Testing results of 5 g samples tested in triplicate and collected from the 14 samples (1-14) presented in table 4.

			PRRSV NA		SVA		PEDV	
Ingredient	Replicate	Expected result	CORE	INDI	CORE	INDI	CORE	INDI
1	1	Pos	34.2006	ND	33.4882	34.6761	38.5302	ND
	2		34.0889	36.8919	32.8025	33.3472	35.9042	ND
	3		33.5307	36.5154	32.6576	33.0037	35.5346	ND
2	1	Pos	34.0893	35.6517	33.287	32.6198	37.4796	ND
	2		32.3417	35.2898	32.0207	32.5304	36.4474	ND
	3		33.2727	35.6525	32.2345	33.163	37.8346	37.4515
3	1	Pos	34.2286	37.6223	35.4056	ND	33.6535	ND
	2		34.1742	36.9313	35.2814	ND	33.5585	38.634
	3		34.2593	35.9496	35.7048	37.3143	34.4505	ND
4	1	Pos	31.5306	34.5428	33.7013	37.303	32.1292	36.4791
	2		31.4605	34.0979	33.6302	36.5724	31.3795	35.4909
	3		31.3705	33.7428	33.9912	ND	31.9641	37.0772
5	1	Pos	ND	ND	35.5418	34.3574	ND	ND
	2		ND	ND	35.021	35.7474	ND	ND
	3		ND	ND	35.8355	35.385	ND	ND
6	1	Pos	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	3		ND	ND	ND	ND	ND	ND
7	1	Neg	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	3		ND	ND	ND	ND	ND	ND
8	1	Pos	32.4224	36.8638	28.249	28.3866	ND	ND
	2		32.8022	35.1648	29.0602	29.3468	38.8057	ND
	3		33.4826	36.1916	29.3574	29.9549	ND	ND
9	1	Pos	36.0103	41.2232	34.9557	36.9654	ND	ND
	2		34.6146	39.7062	34.0309	34.8713	37.6832	ND
	3		35.5754	39.1211	34.6784	35.3293	37.2351	ND
10	1	Pos	27.8312	31.0334	32.3752	37.0265	32.207	38.5394
	2		27.2172	30.5881	31.85	38.0079	31.2656	36.4828
	3		28.3415	31.5046	33.5862	37.0393	32.9962	38.4278
11	1	Pos	34.8472	39.0014	35.2175	ND	34.6082	38.5276
	2		35.0263	37.9161	36.1787	38.081	33.6364	38.1356
	3		32.5701	36.3324	34.7657	36.4331	32.5854	ND
12	1	Pos	ND	ND	ND	ND	ND	ND
	2		ND	ND	38.1526	37.1897	ND	ND
	3		ND	ND	ND	ND	ND	ND
13	1	Pos	ND	ND	35.5716	34.5633	ND	ND
	2		ND	ND	35.2732	36.3082	ND	ND
	3		ND	ND	35.946	36.0306	ND	ND
14	1	Neg	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	3		ND	ND	ND	ND	ND	ND

The diagnostic sensitivity of the different extraction methods performed in the 5g samples was compared. From the 14 1-lb feed samples we received from Dr. Dee, we weighed out 5 g samples and performed independent nucleic acid extractions using the CORE and the IndiMag kits and testing of these samples was performed with the Tetracore, EZ-PRRSV, EZ-SVA and EZ-PEDV assays. In **Table 4** bellow, we provide a summary of the number expected results for each target virus and the actual number of positive and negative samples detected in the original VDL that tested these samples and after the samples were extracted with MagMax Core or the IndiMag kits. The two negative control samples tested negative at Cornell AHDC for all three viruses and after extraction with both CORE and IndiMag kits.

These results from these studies were used to estimate the sensitivity of the extraction methods used in randomly weighed 5 g samples. As shown in **Table 5**, extraction with the MagMax CORE kit resulted in higher sensitivity for all target viruses (87.5% for PRRSV, 95.83% for SVA and

79.17% for PEDV) when compared to the other methods used at AHDC (IndiMag and MVP) and to the method used in the VDL that originally tested these samples.

Table 5 – Estimates of sensitivity of CORE or IndiMag extraction in randomly collected 5 g feed samples.

		PRRSV NA		SVA		PEDV	
	Expected	CORE	INDI	CORE	INDI	CORE	INDI
Pos	12	8	8	11	11	8	5
Neg	2	4	4	3	3	4	7

2x2

	CORE				INDI		
PRRSV	8	0	8	**PRRSV**	8	0	8
	4	2	6		4	2	6
	12	2			12	2	
Se	66.67				66.67		
Sp	100				100		

	CORE				INDI		
SVA	11	0	11	**PRRSV**	11	0	11
	3	2	5		3	2	5
	12	2			12	2	
Se	91.67				91.67		
Sp	100				100		

	CORE				INDI		
PEDV	8	0	8	**PRRSV**	5	0	5
	4	2	6		7	2	9
	12	2			12	2	
Se	66.67				41.67		
Sp	100				100		

This study performed a side-by-side comparison of nucleic acid extraction protocols for use in feed ingredients. Results presented here show that the MagMax Core extraction kit is efficient in extracting viral nucleic acid from feed ingredients. This extraction kit outperformed the extraction obtained with the use of IndiMag and MVP II extraction kits. Additionally, extraction performed with the MagMax core kit was also better than extraction performed at another VDL (MagMax Viral RNA kit). Extraction with MagMax Core resulted lower RT-PCR Ct values for a least 2 of the 3 viruses tested and in higher sensitivity. The results obtained in this study are encouraging and reveal efficient pathogen nucleic acid extraction from conventional and organic soybean meal and from complete swine feed.

One of the main issues that likely remain to be addressed regarding feed testing is the sampling method and sample processing. Our data obtained in samples processed in large batched (1 lb with 1L of PBS) (Table 1) show a slightly better performance of the RT-PCRs for PRRSV, SVA and PEDV in these samples, when compared to the tests performed in the same sample, that were processed in small 5 g batches. This becomes even a bigger challenge when considered in the context of the volume of feed and feed ingredients that are transported from mill to farm daily.

The result of the present project shed some light into the

Publications, presentations or abstracts of the project results:

1. AASV Annual meeting 2022. Work will be presented at the 2022 ASV meeting.