

# **Controlled Experiments Provide Conclusive Evidence Spray-Dried Porcine Plasma is a Safe Ingredient and is Not a Source of Infective PED Virus**

***Research Report by the North American Spray Dried Blood and  
Plasma Producers Association (NASDBPP)***

Porcine Epidemic Diarrhea virus (PEDv) is difficult to control and causes high death loss in suckling pigs less than two weeks old resulting in significant financial loss to all sectors of the swine industry. PEDv spreads quickly and easily. The primary route of infection is through direct contact with infected pigs or from the manure of infected pigs. Other routes of infection responsible for spreading the virus may be contaminated transport vehicles, farm equipment and farm workers and visitors. Industry leaders are actively discussing and reviewing data concerning the role of feed and feed ingredients in the spread of PEDv. Speculation that PEDv is spread by feed has led to implementation of costly bio-security programs, often with little controlled research or data supporting the necessity or effectiveness of the program.

The first report of PEDv in Ontario, Canada and the subsequent investigation resulted in the belief that nursery feed containing porcine plasma may be the source of PEDv infections. The CFIA reported infective virus was detected in samples of porcine plasma but infective virus could not be detected in the feed containing the porcine plasma. Even with this conflicting data, many industry professionals concluded spray-dried porcine plasma is spreading the disease. In addition, and despite the long history of indisputable performance benefits, some veterinarians have recommended removing spray-dried porcine plasma, and in some cases all porcine-based ingredients, from feed for swine.

Epidemiology is a powerful scientific tool that can be used to identify associations of exposure to health outcomes. Epidemiologic observation allows scientists to form a hypothesis and then the hypothesis can be tested in controlled experiments.

## EXECUTIVE SUMMARY

Independently, NASDBPP and FDA conducted controlled experiments to test the hypothesis that spray-dried porcine plasma may contain infective PEDv. The results of these experiments support the conclusion that spray-dried porcine plasma is a safe feed ingredient. The manufacturing process under industry standards inactivates PEDv. However, like any feed ingredient, post-processing contamination is a constant risk and may be the cause of the infective PEDv found on porcine plasma as reported by the CFIA.

<b>Hypothesis: If PCR+ spray-dried porcine plasma contains infective PEDv and is a vector spreading PED:</b>	
<b>Then:</b>	<b>Results of controlled experiments:</b>
1: PEDv will survive spray drying.	1: Spray drying inactivated PEDv.
2: Retained samples of plasma investigated by CFIA in bioassay will contain infective PEDv.	2: Retained plasma samples subjected to bioassay studies by FDA and NASDBPP were not infective for PEDv.
3: Nursery feed containing PEDv PCR+ plasma should infect pigs.	3: Two independent studies using 21-day-old weaned pigs fed 5% PEDv PCR+ plasma in a meal diet for 21 or 28 days post-weaning did not infect pigs with PEDv.
4: Spray-dried plasma contaminated post-processing with PEDv should survive through distribution until consumed by pigs.	4: PEDv inoculated on spray-dried plasma did not survive by: 7 days stored at 71°F (22°C) 14 days stored at 54°F (12°C) 21 days stored at 39°F (4°C)
5: Pigs in a PEDv-free region fed PEDv PCR+ plasma from the US should become infected with PEDv.	5: Millions of pigs in Brazil and Western Canada fed PEDv PCR+ porcine plasma imported from the US since last summer have not developed PEDv.
<b>Conclusion:</b>	
<ul style="list-style-type: none"> <li>• Experimental results show that PCR+ spray-dried porcine plasma does not contain infective PEDv.</li> <li>• Infective PEDv reported by CFIA on samples of spray-dried porcine plasma collected from the field is likely the result of post-processing contamination.</li> </ul>	

Spray-dried porcine plasma investigated by CFIA did not contain infective PEDv when it left the plant. Post-processing contamination may have resulted in the detection of infective PEDv in the samples collected by CFIA.

## RESEARCH AND EXPERIMENTS:

### I. The Effect of Spray Drying Conditions on the Survival of PEDv.

**Hypothesis: If spray dried porcine plasma contains infective virus and is a vector spreading PEDv, then the virus will survive the spray drying process.**

Liquid bovine plasma was inoculated with PEDv (Strain CV777) to a final TCID<sub>50</sub> (median tissue culture infective dose) concentration of 10<sup>4.2</sup> per ml of liquid plasma. Three aliquots of the PEDv-inoculated plasma were spray-dried using a laboratory scale Büchi 190 Mini Spray Dryer at an inlet temperature of 200°C (392°F). In order to simulate manufacturing conditions for commercial spray-dried plasma, the product was processed to achieve an inner temperature of 70°C or 80°C throughout its substance, respectively. The normal outlet temperature of product at commercial manufacturing plants is 80°C throughout its substance. The test also included samples processed with a lower outlet temperature to determine if PEDv could remain active at 70°C. Both liquid and spray-dried samples were analyzed for PEDv infectivity in VERO cell monolayers using a micro-titer assay procedure and were subjected to 3 consecutive serial passages to new VERO cell culture. The PEDv strain CV777 was used in this study because it is known to multiply on VERO cells to a high enough concentration to determine inactivation by spray drying.

Spray drying condition <sup>1</sup>	Tissue culture infectivity of spray-dried plasma
Processing @ 70°C throughout substance	Not detected <sup>2</sup>
Processing @ 80°C throughout substance	Not detected <sup>2</sup>

<sup>1</sup> Inlet temperature was 200°C.

<sup>2</sup> Estimated reduction of PEDv infectivity in spray-dried plasma was calculated to be > 10<sup>5.15</sup> TCID<sub>50</sub>/g. There were three samples per spray drying condition. Each sample was subjected to three serial passages.

These results show that PEDv is inactivated by spray drying at temperatures even lower than those used for commercial production of spray-dried plasma.

These results do not support the conclusion that spray-dried porcine plasma contains infective PEDv and is a vector spreading PED.

## II. Bioassays and Feeding Trials Designed to Determine if Spray-Dried Porcine Plasma PCR+ for PEDv Contains an Infective Virus.

**Hypothesis: If spray-dried porcine plasma contains infective virus and is a vector spreading PEDv, then the retained samples of spray-dried plasma samples being investigated by CFIA should also contain infective PEDv.**

Because the spray-dried plasma product investigated by CFIA was produced in the United States, the CFIA requested the FDA to conduct an investigation. The FDA inspected the US spray-dried plasma manufacturing plant, reviewed production records (including the production records of the spray-dried plasma CFIA investigated) and collected retained samples of the specific lot of spray-dried plasma in question. The production records confirmed the specific lot of spray-dried plasma was produced within normal operating standards.

Independently, both the FDA and NASDBPP submitted the retained spray-dried plasma samples for further analysis including pig bioassays. The FDA recently released the results of their pig bioassays in which three retained samples from the lot of plasma being investigated by CFIA were tested for the presence of infective PEDv. All three spray-dried plasma samples were negative for PEDv infectivity by bioassay.

### Results of FDA Bioassay

Spray-dried plasma	Sample PCR	Bioassay <sup>1</sup>	
		PCR	Serology
Sample 1	Positive	Negative	Negative
Sample 2	Positive	Negative	Negative
Sample 3	Positive	Negative	Negative

<sup>1</sup> Three spray-dried porcine plasma samples corresponding to the CFIA samples were prepared and inoculated into 17-19 day old pigs (5 pigs/sample).

In addition the NASDBPP conducted additional pig bioassays on spray-dried porcine plasma. Two samples from the same lot investigated by CFIA and two samples from randomly selected production lots were tested. All four samples of spray-dried plasma were negative for PEDv infectivity by bioassay.

## Results of NASDBPP Bioassay

Sample ID	Initial PEDv PCR (CT)	Clinical Signs 72 hr post inoculation <sup>1</sup>			Jejunum PEDv RT-PCR CT			IHC <sup>2</sup>
		Pig 1	Pig 2	Pig 3	Pig 1	Pig 2	Pig 3	
Spray-dried plasma								
Sample 1 <sup>3</sup>	31.28	0	0	0	Neg.	Neg.	Neg.	Neg.
Sample 2 <sup>3</sup>	29.86	0	0	0	Neg.	Neg.	Neg.	Neg.
Sample 3 <sup>4</sup>	29.86	0	0	1	Neg.	Neg.	Neg.	Neg.
Sample 4 <sup>4</sup>	30.71	0	0	0	Neg.	Neg.	Neg.	Neg.
PEDv Challenge								
Yes	18.61	3	3	3	16.53	15.30	15.30	Pos.
No	-	0	0	0	Neg.	Neg.	Neg.	Neg.

<sup>1</sup> Score 0 = no clinical signs well-formed solid feces; score 1= soft poorly formed feces; score 2= presence of watery liquid feces; score 3= watery diarrhea and dehydration.  
<sup>2</sup> IHC = Immunohistochemistry.  
<sup>3</sup> Samples 1 and 2 were from the retained sample of the spray dried plasma in the CFIA investigation.  
<sup>4</sup> Samples 3 and 4 were randomly selected commercial spray-dried plasma product.

In contrast to the results reported by CFIA, the FDA and the NASDBPP were unable to detect infective PEDv in retained samples of porcine plasma. It is important to note that the retained samples were stored under the same conditions as the commercial product. However, CFIA collected the plasma samples from the field after the plasma had been picked up by the customer and delivered to the feed mill, while the retained samples were maintained in the spray drying plant warehouse.

These results do not support the conclusion that spray-dried porcine plasma contains infective PEDv and is a vector spreading PED.

Some in the industry have questioned the sensitivity of pig bioassays because they involve a small number of animals fed a limited amount of test ingredient. Therefore, it is difficult to detect a low occurrence event and a negative does not necessarily mean negative. In an attempt to address this concern, two additional trials were designed with feeding protocols reflecting industry practice.

**Hypothesis: If spray-dried porcine plasma contains infective virus and is a vector spreading PEDv, then feeding plasma containing diets for longer periods of time, similar to commercial practice, will increase the chance of detecting the presence of infective virus.**

**Feeding Trial 1:** At a contract research facility that had previously housed nursery pigs tested and found negative for PEDv, 48 pigs weaned at an average of 21 days old were randomly distributed into two different treatment groups with 6 pens per dietary treatment and 4 pigs per pen. The groups were as follows:

- Treatment 1 - Pigs received a control diet without spray-dried porcine plasma.
- Treatment 2 - Pigs received a meal diet containing 5% commercial spray-dried porcine plasma that was PCR+ for PEDv (spray-dried porcine plasma, Ct = 26.2; Complete feed, Ct = 30.1).

Basal diets were similar to standard commercial diets for weaned pigs and 5% spray-dried porcine plasma was included in treatment 2 replacing soy protein concentrate on an equal lysine basis. The spray-dried porcine plasma used in the test diet had the lowest Ct result of commercial product produced during 2014. Experimental diets were fed in a meal form (eliminating the confounding heat treatment associated with pelleting) for 14 days after weaning and then all pigs were fed the control diet for an additional 7 days.

Pigs were evaluated daily for clinical symptoms of both enteric and respiratory diseases. Pens and pigs were evaluated daily and assigned a score for diarrhea. Rectal fecal swabs were collected from each pig at day 0, 3, 7, 14, and 21 and immediately submitted for PEDv PCR analysis. Pigs were necropsied on day 21. Gross evaluation of tissues was conducted to determine if any abnormalities were noted. Intestinal contents were collected at termination and submitted for PEDv PCR and tissue samples were subjected to IHC. Terminal blood samples were collected and submitted for PEDv antibody analysis. In this experiment, feeding pigs a diet containing 5% commercial spray-dried porcine plasma that was PCR+ for PEDv did not demonstrate any evidence of PEDv infectivity in these pigs through 21 days post-weaning.

### Results of nursery feeding Study 1

Analysis	Trt. 1 - Control	Trt. 2 –Spray-dried plasma
PCR in fecal samples at day 0	Negative	Negative
PCR in fecal samples at day 3	Negative	Negative
PCR in fecal samples at day 7	Negative	Negative
PCR in fecal samples at day 14	Negative	Negative
PCR in fecal samples at day 21	Negative	Negative
PCR in intestinal tissue at day 21	Negative	Negative
Immunohistochemistry at day 21	Negative	Negative
Serum antibodies against PEDv at day 21	Negative	Negative

**Feeding Trial 2:** At Iowa State University, 21-day-old weaned pigs confirmed negative for PEDv were randomly allotted to four treatment groups as follows:

- Treatment 1: Not inoculated with PEDv and fed a control meal form diet without spray-dried porcine plasma (SDPP).
- Treatment 2: Not inoculated with PEDv and fed a meal form diet with 5% SDPP that was PCR+ for PEDv (Ct=30.0).
- Treatment 3: Inoculated with PEDv and fed the control diet.
- Treatment 4: Inoculated with PEDv and fed the diet containing 5% SDPP that was PCR+ for PEDv (Ct=30.0).

Diets for Treatment 1 and 2 were fed for 28 days post-weaning. Diets for Treatment 3 and 4 were provided to pigs 4 days before PEDv (isolate 13-19338E) inoculation, then fed for an additional 28 days. There were 6 pigs allotted per treatment. Three pigs per group were necropsied on day 7 and 28 of the study.

## Results of nursery feeding Study 2

Treatment	1	2	3	4
Diet	Control	5% SDPP	Control	5% SDPP
PEDv Inoculated	No	No	Yes	Yes
PEDv PCR feces d 0	Neg	Neg	Neg	Neg
PEDv PCR feces d 3	Neg	Neg	Pos	Pos
PEDv PCR feces d 7	Neg	Neg	Pos	Pos
PEDv PCR feces d 14	Neg	Neg	Pos	Pos
PEDv PCR feces d 21	Neg	Neg	Pos	Pos
PEDv PCR feces d 28	Neg	Neg	Pos	Pos
PEDv Serum antibodies d 28	Neg	Neg	Pos	Pos

Regardless of diet fed, pigs in Treatment 1 and 2 not inoculated with PEDv did not develop PEDv as determined by PCR of fecal swab samples or by development of serum antibodies against PEDv. Feeding non-inoculated pigs a diet with PEDv PCR+ SDPP did not result in PEDv infectivity over the 28-day study. All pigs in treatments 3 and 4 inoculated with PEDv, regardless of diet fed, had PCR positive fecal samples starting at day 3 post-inoculum through day 28 and had developed serum antibodies against PEDv.

The data from these two studies demonstrates that pigs fed a diet containing spray-dried porcine plasma PCR+ for PEDv simulating commercial feeding programs did not become infected with PEDv.

These results do not support the conclusion that spray-dried porcine plasma contains infective PEDv and is a vector spreading PED.

### III. Stability Trials Designed to Determine the Survival on Dry Plasma Stored at Different Temperatures.

**Hypothesis: If spray dried porcine plasma contains infective virus and is a vector spreading PEDv, then PEDv should survive on spray-dried plasma long enough to move through the commercial feed channel**

Spray-dried bovine plasma was inoculated with PEDv strain CV777 to a final concentration of  $10^3$  TCID<sub>50</sub>/g and maintained at room temperature (20-22°C) for 1 week. Initial and final samples were assayed in VERO cell culture as previously described. At 7 days, PEDv infectivity was not

detected in the inoculated spray-dried bovine plasma. In summary, PEDv did not survive on spray-dried plasma by 7 days when stored at room temperature.

## Results

<b>PEDv inoculated on spray-dried bovine plasma held at room temperature (20-22°C)</b>	
Day of Storage	Virus Isolation Result <sup>1</sup>
0	Positive
7	Negative
<sup>1</sup> Each storage time was titrated for PEDv growth in five flasks (75 cm <sup>2</sup> ) on VERO cells with 3 different passages. At day 0, PEDv concentration on spray-dried plasma was 1x10 <sup>3</sup> TCID <sub>50</sub> /g. A positive result indicates presence and a negative result indicates absence of viable PEDv.	

Based on the results from the initial experiment, a follow-up experiment was designed to understand the effects of storage time at various temperatures on PEDv survival on spray-dried plasma. In this experiment, spray-dried bovine plasma was externally contaminated with PEDv and stored at 4°C, 12°C and 22°C. PEDv isolation, as described previously, was determined at day 0, 7, 14, and 21 post-inoculum representing 5 samples per time by temperature storage condition.

## Results:

<b>PEDv survival on spray dried bovine plasma held at various time by temperature conditions<sup>1</sup></b>			
<b>Day of storage</b>	<b>Storage temperature</b>		
	<b>4°C/39°F</b>	<b>12°C/54°F</b>	<b>22°C/71°F</b>
<b>0</b>	Positive	Positive	Positive
<b>7</b>	Positive	Positive	Negative
<b>14</b>	Positive	Negative	Negative
<b>21</b>	Negative	Negative	Negative
<sup>1</sup> Each condition was titrated for virus growth in five flasks (75 cm <sup>2</sup> ) on VERO cells with 3 different passages. At day 0, PEDv concentration on spray-dried plasma was 1x10 <sup>2.8</sup> TCID <sub>50</sub> /g. A positive result indicates presence and a negative result indicates absence of viable PEDv.			

Summary:

- At 4°C storage temperature, PEDv survived on spray-dried plasma for 14 days but did not survive when stored for 21 days.
- At 12°C storage temperature, PEDv survived on spray-dried plasma for 7 days but did not survive when stored for 14 or 21 days.
- At 22°C room temperature storage, PEDv did not survive on spray-dried plasma for 7, 14 or 21 days.

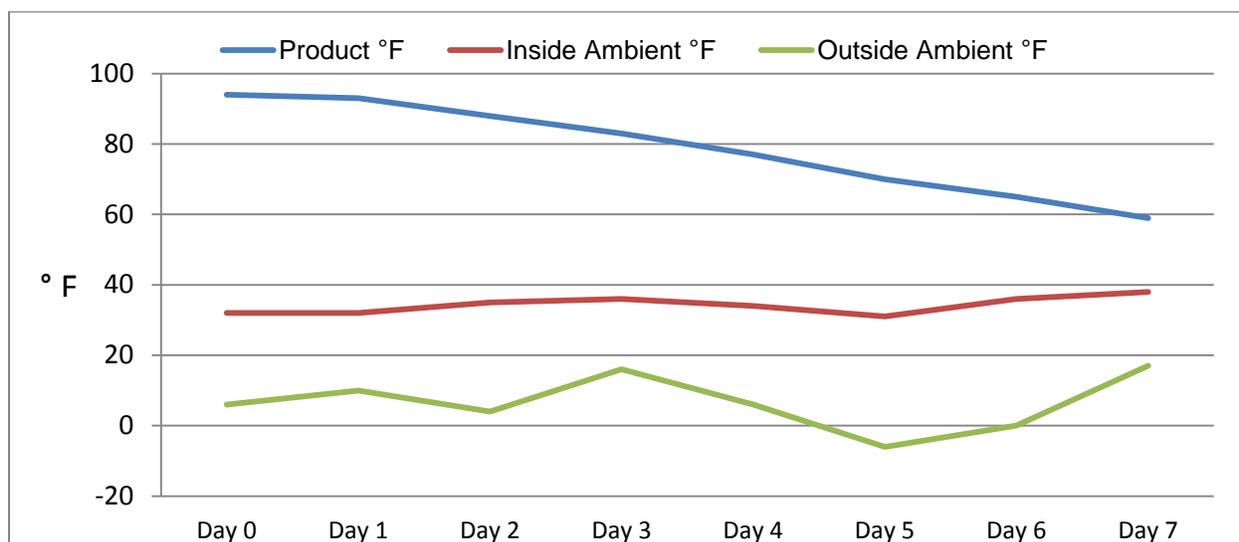
PEDv does not survive for extended periods of time on spray-dried plasma. In two experiments, PEDv survived less than 7 days when held at room temperature (71°F). Even at refrigerator temperatures, PEDv survived less than 21 days. Typically, spray-dried plasma will be held for a minimum of 1 week before testing is complete and the product is released for sale. Depending on inventory, another 2 weeks will be required for product to be sold, picked up, delivered and mixed into complete feed. In addition, these data support the redundant safety step introduced by the NASDBPP of holding spray-dried plasma for 14 days at 71°F before release for sale.

These results do not support the conclusion that spray dried porcine plasma contains infective PEDv and is a vector spreading PED.

Typically, spray-dried plasma was stored in an unheated warehouse before shipment to the feed manufacturer. Because of the exceptionally cold winter of 2013 / 2014, it has been speculated the spray-dried plasma was essentially held in a “deep freeze” effectively extending the survival of PEDv and therefore allowing spray-dried plasma to spread PEDv. The NASDBPP conducted the following study to understand actual storage conditions in warehouse systems. However, the data from the study indicated this speculation is incorrect.

During late February of 2014, the temperature of spray-dried porcine plasma was measured during packaging and daily while the product was held for QA analysis (Figure 1). The warehouse, located in central Iowa, was not insulated or heated. The outside temperatures ranged between -6°F to 17°F (-21.1°C to -8.3°C) while the warehouse temperature fluctuated between 27°F to 37°F (-2.8°C to 2.8°C). At packaging, the spray-dried plasma was approximately 95°F and slowly cooled to 59°F within 7 days. The product would not be expected to cool below the warehouse temperature.

Figure 1. Temperature evolution of one ton totes of spray-dried porcine plasma stored in an unheated warehouse in Iowa during February 2014.



Therefore, even in an uninsulated warehouse during the exceptionally cold 2013/2014 winter, spray-dried plasma did not experience a "deep freeze". Actually, spray-dried plasma maintained temperatures shown to inactivate PEDv.

#### IV. Incidence of PED in Distinct Regional Swine Populations that Consumed Significant Quantities of Spray-Dried Porcine Plasma PCR+ for PEDv.

**Hypothesis: If spray-dried porcine plasma contains infective virus and is a vector spreading PEDv, then feeding spray-dried porcine plasma PCR+ for PEDv should spread PED to regions free of PED.**

A spray-drying facility located in the southern region of Brazil is dedicated to porcine blood processing only. Samples of spray-dried porcine blood were collected from production lots dried in late December 2013 and early January 2014. The samples were submitted for PCR analysis for the presence of the PEDv genome. The samples were negative confirming the observations of Brazilian veterinarians that PEDv has not entered Brazil. During 2013, enough US-produced spray-dried porcine plasma (PCR positive for PEDv) was exported to Brazil to feed between 2.5 to 3.5 million pigs. In spite of this large quantity of US-sourced PEDv PCR+ spray-dried porcine plasma being fed in Brazil, PEDv has not been diagnosed or reported in this swine population.

<b>Spray-dried porcine plasma produced in Brazil<sup>1</sup></b>	
<b>Manufacturing date</b>	<b>PEDv PCR result</b>
December 29, 2013	Negative
December 31, 2013	Negative
January 3, 2014	Negative
January 7, 2014	Negative
January 8, 2014	Negative
<sup>1</sup> Each single lot of spray-dried porcine plasma was estimated to have been produced from the blood of approximately 100,000 Brazilian pigs.	

Additionally, a spray-drying facility located in Western Canada is dedicated to porcine blood processing only. Samples of spray-dried porcine plasma were submitted for PCR analysis for the presence of the PEDv genome. All samples analyzed over the last several months have been PCR Negative. Between April 2013 and December 2013, enough PEDv PCR+ spray-dried porcine plasma was exported from US spray-dried plasma manufacturing plants into Western Canada to feed between 3.5 million to 4.0 million pigs. The US-sourced spray-dried porcine plasma was PCR+ for PEDv. PEDv cases were not detected in Western Canada during that time period. The pig population in Western Canada has remained negative for PEDv through March 2014 with the exception of one case reported in February that was determined unrelated to feed.

<b>Spray-dried porcine plasma produced in Western Canada<sup>1</sup></b>	
<b>Manufacturing date</b>	<b>PEDv PCR result</b>
August, 2013	Negative
September, 2013	Negative
October, 2013	Negative
November, 2013	Negative
December, 2013	Negative
January, 2014	Negative
February, 2014	Negative
March, 2014	Negative
<sup>1</sup> Spray-dried porcine plasma produced from slaughter pigs in Western Canada were negative for PEDv even though PEDv PCR+ spray-dried porcine plasma from the US had been fed in Canada since early summer of 2013.	

Data from controlled experiments and the regional observations from feeding PCR+ spray-dried porcine plasma from the US to 2.5 to 3.5 million Brazilian pigs and between 3.5 to 4.0 million pigs in Western Canada do not support the conclusion that spray-dried porcine plasma contains infective PEDv and is spreading the disease.

## **SUMMARY**

Spray-dried plasma is a safe effective feed ingredient. It contains a diverse mixture of proteins providing nutrition to support beneficial and functional biological effects on the ability of pigs to thrive and cope with post-weaning stress. The nutrition provided by spray-dried plasma as a key ingredient in nursery diets has been recognized by the swine industry worldwide because of its well-documented beneficial effects on post-weaning growth, feed intake, morbidity indices, and survival. In fact, results of many studies conclude that nutrition supplemented with spray-dried plasma supports and maintains swine under challenge with PCV2-SD, PRRSv, and other respiratory and gastrointestinal diseases, including TGEv. Therefore, it can be expected that feed supplemented with spray-dried plasma has potential to support the ability of pigs to cope with PEDv.

The recent research and study results outlined above demonstrate that PEDv is inactivated during commercial production of spray-dried plasma and PEDv PCR+ spray-dried porcine plasma is not a source of PEDv infectivity. These results confirm the evidence from past research that spray-dried porcine plasma is a safe and vital feed ingredient for the global swine industry.