# Estimated quantity of swine virus genomes based on quantitative PCR analysis in spray-dried porcine plasma samples collected from multiple manufacturing plants.

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Short title: Quantitation of viral genomes in porcine plasma collected from abattoirs

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# Abstract (max 300 words)

This survey was conducted to estimate the incidence and level of potential viral contamination in commercially collected porcine plasma. Samples of spray dried porcine plasma (SDPP) were collected over a 12- month period from eight spray drying facilities in Spain, England, Northern Ireland, Brazil, Canada, and the United States. In this survey, viral load for several porcine pathogens including SVA, TGEV, PRRSV (EU and US strains), PEDV, PCV2, SIV, SDCoV and PPV were determined by qPCR. Regression of Ct on TCID50 of serial diluted stock solution of each virus allowed the estimate of potential viral level in SDPP and unprocessed liquid plasma (using typical solids content of commercially collected porcine plasma). In this survey SVA, TGEV or SDCoV were not detected in any of the SDPP samples. Brazil SDPP samples were free of PRRSV and PEDV. Samples of SDPP from North America primarily contained the PRRSV-US strain while the European samples contained the PRRSV-EU strain (except for one sample from each region containing a low estimated level of the alternative PRRSV strain). Estimated viral level tended to be low ranging from $<1.0$ $\log_{10}$ $TCID_{50}$ to $<2.5$ $\log_{10}$ $TCID_{50}$. Estimated level of SIV was the exception with a very low incidence rate but higher estimated viral load $<3.9$ $\log_{10}$ $TCID_{50}$. In summary, the incidence of potential viral contamination in commercially collected porcine plasma was variable and estimated virus level in samples containing viral DNA/RNA was low.


**Keywords**; spray-dried porcine plasma; swine viruses; qPCR; Worldwide viruses distribution

# Introduction

Spray dried porcine plasma (SDPP) is a complex mixture of functional components including immunoglobulins, albumin, transferrin, fibrinogen, lipids, growth factors, bioactive peptides, enzymes, hormones, and amino acids commonly used in feed for young animals including pigs, calves, and poultry [1-4].

It has been speculated that the use of SDPP in swine feed contributed to the spread of infective viruses such as *Porcine circovirus 2* (PCV-2) and *Porcine epidemic diarrhea virus* (PEDV) [5-7]. However, other evidence demonstrates that reduced mortality and morbidity is associated with the use of SDPP in pig diets [1, 3, 8, 9] and experimental and epidemiological evidence demonstrate that SDPP does not spread diseases [10-12].

The manufacturing process to produce SDPP includes multiple hurdles that have been validated to inactivate potential viral contamination. These hurdles include spray drying (SD, 80$^{o}$C throughout substance), ultraviolet light (UV) treatment (3000 J/L) and post drying storage (PDS) at 20$^{o}$C for 14 d [13-19]. Depending on the virus, the theoretical cumulative inactivation for SD and PDS range from 5.8 to 9.1 $\log_{10}$ $TCID_{50}$/g liquid plasma, while SD, PDS and UV range from 11.7 to 20.9 $\log_{10}$ $TCID_{50}$/g liquid plasma (Table 1). The World Health Organization recommended cumulative robust inactivation procedures capable to eliminate 4 $\log_{10}$ of virus by each of these steps in the manufacturing process for human blood and plasma products [20, 21].

While the inactivation capacity of the multiple hurdle manufacturing process has been validated for several economically important swine viruses, it is also important to estimate the potential virus quantity in liquid plasma used to produce SDPP. Therefore, this

survey was conducted to estimate the quantity and determine the frequency of genome detection of different swine viruses in commercially produced SDPP samples collected from 8 different manufacturing plants. Results obtained from quantitative polymerase chain reaction (qPCR) analyses of the SDPP samples were used to infer the potential viral contamination in the liquid porcine plasma from which it was produced.

# Material and Methods

## Ethical statement

No animals were used for the study conducted.

## Spray-dried porcine plasma sample collection

One sample per month was collected from a randomly selected commercial lot of SDPP during 12 consecutive months from eight different manufacturing plants located in Iowa, USA (IA-USA), North Carolina, USA (NC-USA), Santa Catarina, Brazil (SC-Brazil), central Spain (C-Spain), northeastern Spain (NE-Spain), central England (C-England) and Northern Ireland (N-Ireland). The N-Ireland manufacturing plant collect porcine blood from abattoirs located both, in Republic of Ireland and Northern Ireland. Samples from a manufacturing plant located in Quebec, Canada (QB-Canada), were taken biweekly during a 6 month-period.

Samples were collected from July 2018 to June 2019 (SC-Brazil), August 2018 to July 2019 (IA-USA, NE-Spain, C-Spain and N-Ireland) or September 2018 to August 2019

(NC-USA, C-England). The QB-Canada plant provided 12 samples randomly collected from March to August 2019. The collected SDPP samples represented a single point in time, not the entire month. Whole blood or plasma was stored at the abattoir in agitated tanks, transported to the spray drying facility in dedicated tankers and stored and blended with other plasma from different slaughterhouses in agitated silos before drying. In the manufacturing plants used in this study, a manufacturing lot of SDPP can range between 3,000 to 30,000 Kg of plasma depending on the plant. Therefore, one lot of SDPP represented between 16,650 to 166,500 pigs. During the 12-month collection period, samples were stored in whirl packs (Whirl-Pak®, Nasco, Madison, WI) and held at each plant in the quality assurance laboratory (room temperature) during the collection period. Subsequently, all SDPP samples from each manufacturing plant were sent to the IRTA-CReSA Animal Health Research Center in Barcelona, Spain, and stored until analyses for virus genome. One sample collected in December from the IA-USA plant was damaged during transport and was not used for analysis. Therefore, a total of 95 SDPP samples were analyzed.

## Sample analysis by PCR

All SDPP samples were re-solubilized in distilled water at the ratio 1:9 of SDPP: water volume to represent the typical solid content in liquid plasma. Two hundred milliliters of diluted plasma sample were used for nucleic acid extraction using MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific, MA, USA). The recommended quantity of purified nucleic acids were amplified using real time PCR kits for PCV-2 (LSI VetMAX™ Porcine Circovirus Type 2 Quantification, Thermo Fisher Scientific, MA, USA), *Porcine reproductive and respiratory syndrome virus* [PRRSV] European and North American

strains (LSI VetMAX™ PRRSV EU/NA Real-Time PCR Kit; Thermo Fisher Scientific, MA, USA), *Swine influenza virus* [SIV] (EXOone Influenza A, EXOPOL, Zaragoza, Spain), *Porcine parvovirus* [PPV] (VetMAX™ Porcine Parvovirus Kit, Thermo Fisher Scientific, MA, USA), PEDV, *Transmissible gastroenteritis virus* [TGEV] and Swine deltacoronavirus [SDCoV] (VetMAX™ PEDV/TGEV/SDCoV, Thermo Fisher Scientific, MA, USA) and *Senecavirus A* [SVA] (EXOone Seneca Virus Valley, EXOPOL, Zaragoza, Spain).

According to all PCR kit guidelines, virus genome results with Ct values >40 were considered negative.

## Virus stock production for development of standard curves to convert PCR Ct to $TCID_{50}$/g SDPP.

From those viruses detected in SDPP by qPCR, a stock of each virus was produced in the laboratory. Seven serial dilutions of viral stocks (PEDV, PRRSV-1 (EU strain), PRRSV-2 (US strain), PPV-1, PCV2 and SIV) were analyzed by quantitative PCR/RT-PCR (obtaining the corresponding Ct value) and $TCID_{50}$ titrated. Standard curves were established for each virus by regressing TCID50/g SDPP on Ct results [Fig 1]. Those viral stocks were used as an internal standard on each amplification run/plate and quantitative PCR/RT-PCR Ct values extrapolated to TCID50. Potential viral quantity determined on SDPP was corrected for typical solids content for each commercially collected plasma. TCID50 titers were calculated by the Reed and Muench method [22].

### *Porcine reproductive and respiratory syndrome virus*

*Porcine reproductive and respiratory syndrome virus* 3268 EU strain was propagated in porcine alveolar macrophages (PAM) grown in standard growth media (SGM) containing minimum essential medium eagle (MEM-E; ThermoFisher, Waltham, MA, USA) supplemented with 1% penicillin 10,000 U/mL and streptomycin 10 mg/mL (ThermoFisher), 0.5% Nystatin 10,000 IU/mL (Sigma-Aldrich, Burlington, MA, USA), 1% L-glutamine 200 mM (ThermoFisher) plus 5% fetal bovine serum (FBS). Cells were cultured in 75-$cm^2$ flasks. When cells were confluent, the media was discarded, and the adsorption was done using the virus at 0.01 MOI. After 1.5 hours at 37ºC, inoculum was removed, and 30 mL of medium were added. Titration was done in triplicate obtaining a final titer of $10^{5.48}$ $TCID_{50}$/mL.

*Porcine reproductive and respiratory syndrome virus* RV2332 US strain was propagated in MARC145 cells (ATCC No. CRL-12231) (kindly provided by Dr. Enric Mateu, *Universitat Autònoma de Barcelona*, Barcelona, Spain) using SGM supplemented with 10% FBS as explained above until a viral stock solution with a final titer of $10^{4.95}$ $TCID_{50}$/mL was obtained.

## ***Porcine epidemic diarrhea virus***

*Porcine epidemic diarrhea virus* CV777 strain [23], kindly provided by Dr. Hans Nauwynck (University of Ghent, Belgium), was propagated in VERO cells (ATCC CCL-81) grown in SGM with 10% FBS. Cells were cultured in 175-$cm^2$ flask and when they were confluent, the media was removed, and cells were rinsed twice with PBS. Finally, inoculum was added at 0.001 MOI and adsorption was done for 1 hour at 37ºC. Subsequently, the inoculum was discarded, flasks were rinsed twice with PBS and SGM supplemented with 10

153 mg/mL trypsin, and 0.3% tryptose (Sigma-Aldrich, Burlington, MA, USA). The viral stock
154 was produced in the same cells and was titrated in triplicate obtaining a suspension with a
155 viral titer of $10^{5.42}$ $TCID_{50}$ /mL.

156

157 ## *Swine influenza virus*

158 *Swine influenza virus* strain H1N1 A/Swine/Spain/SF11131/2017 [24] was
159 propagated in MDCK cell line (ATCC CCL-34) grown in DMEM (ThermoFisher, Waltham,
160 MA, USA) supplemented with 1% penicillin (10,000 U/mL), 1% streptomycin (10 mg/mL;
161 ThermoFisher), 0.5% Nystatin (10,000 U/mL) (Sigma-Aldrich, Burlington, MA, USA), 1%
162 L-glutamine 200mM (ThermoFisher) and 5% FBS. Cells were cultured in 175-$cm^2$ flask.
163 When cells were confluent, the media was discarded, and the adsorption was done at 0.1
164 MOI. After 1 hour at 37ºC, inoculum was removed, and 30 mL of medium were added. The
165 viral suspension was titrated in triplicate and the final virus titer was $10^{7.558}$ $TCID_{50}$ /mL.

166

167 ## *Porcine circovirus 2*

168 *Porcine circovirus 2* genotype b isolate Sp-10-7-54-13 [25] was cultured in the PK-
169 15 cell line (provided by the Institute of Virology UE and OIE Reference Laboratory for
170 CSFV, Hannover), grown in SGM with 10% FBS. A mix of 6 mL of virus stock and 7 x $10^6$
171 PK-15 cells resuspended in 50 mL of MEM-E (MOI 0.1) were added in 175 and 25 $cm^2$
172 flasks. At 24 hours cells were treated with glucosamine (Sigma-Aldrich, Burlington, MA,
173 USA) to facilitate the virus infection. Forty-eight hours later, viral infection was checked by

IPMA [26] in the 25 $cm^2$ flask. If more than 25 positive cells were counted in a microscope field, the 175 $cm^2$ flask was trypsinized and the cells were transferred to 3 new 175 $cm^2$ flasks. The virus stock was titrated in triplicate with a final titer of $10^{5.5}$ $TCID_{50}$ /mL.

### *Porcine parvovirus*

*Porcine parvovirus* strain NADL-2 was kindly provided by Dr Albert Bosch (Department of Genetics, Microbiology and Statistics School of Biology, University of Barcelona, Spain). It was propagated in SK-RST cells (ATCC CRL-2842), grown in SGM supplemented with 5% FBS. One mL of virus stock and 9 mL of MEM-E supplemented with 1% pyruvate (Merck KGaA, Darmstadt, Germany) were added to a conical tube with 16 x $10^6$ SK-6 cells and shaken for 30 minutes at 104 rpm and 37ºC. After that time, the contents of the tube were transferred to a 175 $cm^2$ flask, in which 40 mL of MEM-E supplemented with 1% pyruvate were added. A viral suspension was obtained and titrated in triplicate, obtaining a final viral solution of $10^{6.64}$ $TCID_{50}$ /mL.

## Estimation of $TCID_{50}$ and genomic equivalent copies (GEC) from Ct values obtained from q-PCR results.

To establish correspondence of positive qPCR results (measured as Ct values) with $TCID_{50}$/mL and viral genome copy content (GCC), seven serial dilutions of abovementioned titrated virus stocks were performed, and virus genome amplified with a second set of PCR kits (GPS, Genetic PCR Solutions Alicante, Spain). Each kit contained a

195 genome quantified standard for the different viruses tested: PRRSV (PRRSV-I dtec-RT-
196 qPCR, PRRSV-II dtec-RT-qPCR), PEDV (PEDV dtec-RT-qPCR), PPV (PPV-1 dtec-RT-
197 qPCR) and SIV (SIV dtec-RT-qPCR).

198

## 199 Statistical analysis

200 Dilutions of titrated viral stocks were included as an internal standard on each
201 amplification PCR run containing SDPP samples. The Excel software was used to obtain
202 the equation correlating $TCID_{50}$ and Ct values as well as GCC and Ct values. Then, results
203 of the different PCR techniques originally expressed as Ct values for each SDPP sample
204 tested were extrapolated to virus infectious particles and GCC based on the obtained
205 regression formulae.

206 Average, number of observations, standard deviation, minimum value, maximum
207 value, and ranges were calculated within each virus and for each SDPP producing plant
208 using LSMEANS (SAS 9.4, 2016).

209

# 210 Results and Discussion

211 In this survey, viral loads for several porcine pathogens including SVA, TGEV,
212 PRRSV (EU and US strains), PEDV, PCV2, SIV, SDCoV and PPV were determined by
213 qPCR in reconstituted commercial SDPP. First, the Ct values from serial dilutions of a
214 stock solution for each virus allowed the development of a correlation equation between Ct
215 and $TCID_{50}$ that subsequently enabled estimating the putative viral titers in the SDPP

samples. Finally, using typical solids content of unprocessed liquid plasma, the viral level in liquid plasma was adjusted per gram ($TCID_{50}$/g liquid plasma). The relationships between Ct and $TCID_{50}$ of serial diluted stock solutions were linear with a correlation coefficient from 0.95 to 0.995 (Fig 1). Similar correlation coefficients were found when regressing Ct on $log_{10}$ GEC/g on the tested samples (Fig 1). The slope of the lines for either TCID50 or GEC/g were similar, while the intercepts were different (Fig 1), consistent with the fact that not all viral genome copies are infective [27]. In fact, high variability between infectious particles and genome copy numbers were observed among tested viruses, with less than 1 log difference for SIV to around 4 log differences for PCV-2.

Previous research has shown PCR/RT-PCR Ct values in SDPP to be relatively stable during normal storage conditions [19, 28, 29]. Similar levels of viral genome were detected in plasma inoculated with PCV-2 or SIV before and after spray drying (data not shown). The stability of PCR Ct values, the linear relationship between Ct and $TCID_{50}$ and the linear relationship between Ct and GEC provides additional assurance that estimated viral contamination of commercially collected SDPP and estimates of liquid plasma are accurate.

Frequency of detection and estimated quantity of virus in SDPP samples mimicking unprocessed liquid plasma samples collected at different plants is presented in Tables 2 and 3. S1 Table includes Ct values and estimated virus levels reported as $Log_{10}$ GEC/g and $Log_{10}$ $TCID_{50}$/g in reconstituted SDPP from the different manufacturing plants. S2 Table includes the estimated viral levels in unprocessed plasma reported $Log_{10}$ TCID50/g. It is

238 important to recognize that a positive PCR/RT-PCR does not imply infectivity [16], fact
239 that was observed for all the viruses studied in the present work.

240 In this survey neither SVA, TGEV nor SDCoV were detected in any of the SDPP
241 samples (data not shown). SVA infection has been detected in the Americas and Asia, but
242 not in Europe [30], so, it was expectable to have a significant number of RT-PCR negative
243 samples. Importantly, viremia and clinical signs in SVA infected pigs appear within 2 to 3
244 days post-inoculation [31, 32], so, it is very difficult to have pigs in its highest shedding
245 phase without displaying clinical signs. Despite SVA infected animals are sporadically
246 detected on-farm and at abattoirs during ante-mortem inspection [33], effective
247 identification of non-symptomatic animals probably contributed to the absence of SVA
248 genome in the tested SDPP samples. Further supporting this hypothesis, a US survey
249 reported only 1.2% of oral samples from 25 states being RT-PCR positive for SVA [34].
250 On the other hand, the inability to detect TGEV in these samples is also consistent with a
251 very low incidence in the US and European swine population [35-37]. In case of SDCoV,
252 the current data agree with prevalence results from Puente et al. [38] that indicated absence
253 of SDCoV and TGEV in 106 Spanish pig farms analyzed between 2017-2019.
254 Furthermore, Ajayi et al. [39] indicated that the presence of SDCoV in Ontario farms
255 decrease from 1.14% in 2014 to 0.08% in 2016, matching with our results of very low
256 presence of SDCoV in the North American pig population analyzed in 2018-19.
257 Noteworthy, samples from Brazil were negative for both PRRSV and PEDV, which is
258 consistent with other reports indicating that these viruses are not present in this country [40-
259 45].

All SDPP samples were tested for both the EU and US strains of PRRSV independently of the geographical origin of the SDPP. Samples from the US contained PRRSV genotype 2, except for one sample from US-IA that had a PRRSV genotype 1 RT-PCR positive result (Ct of 35.70, equivalent to -0.29 $Log_{10}$ $TCID_{50}$/g SDPP). Similarly, the samples from EU contained the PRRSV genotype 1, except for one sample from Spain-C that had PRRSV genotype 2 positivity (Ct of 36.08, equivalent to -2.09 $Log_{10}$ $TCID_{50}$/g SDPP). The detection frequency of positive samples differed between plants, with 100% in those from US-IA, 17% in US-NC and 50% in Canada production plants. In Europe, the RT-PCR positivity against PRRSV was 33% for Spain-NE, 58% for Spain-C, 50% for England and 83% for N-Ireland. However, in both the US and in the EU, the estimated PRRSV $TCID_{50}$ in SDPP was very low (< 2 virus particle/g SDPP, average Ct of 33.83 ± 1.83 and 33.99 ± 1.26 for genotype 2 and 1, respectively in SDPP samples). Other works have reported low incidence of PRRSV viremia in slaughtered aged pigs [46] and differences in infection prevalence among US geographical areas [47], which would be aligned with the results obtained in the present survey.

Estimated PEDV levels in liquid plasma was low (<2.00 $log_{10}$ PEDV/g SDPP). The detection frequency of positive samples was 82% in US-IA, 50% in US-NC and 8% in Canada. These results indicated that PEDV genome distribution was low in Eastern Canada compared with the USA and agrees with surveillance of PEDV cases reported in North America [48, 49]. In Europe, the incidence of positive PEDV samples was 83% in Spain-NE, and 67% in Spain-C while in England and N-Ireland the samples were negative. Although the present study was not designed to elucidate seasonal differences in the estimated quantity for PEDV genome in the different parts of the world, obtained results

283 suggest higher frequency of detection and viral loads during the winter, while it was lower
284 in summertime (S1 and S2 Tables). These results are in line with the fact that PEDV is
285 more stable in cold environments [50] and has lower incidence of clinical diarrhea cases at
286 farms during the summer season [51].

287 Both PPV and PCV-2 are stable non-enveloped DNA viruses [52, 53]. Frequency
288 of detection of both PPV and PCV-2 was 100%, since all samples tested positive for
289 genetic material. In all regions, the estimated level of PCV2 was low (<2 $\log_{10}$ $TCID_{50}$/g
290 SDPP that correspond to less than 5 virus particle per g of raw plasma), while PPV
291 presence was slightly higher (<2.0 $\log_{10}$ $TCID_{50}$/g liquid plasma). Other studies have
292 reported low levels of PCV-2 viremia in finishing swine [54, 55], in part due to the
293 widespread use of PCV-2 vaccine [56, 57]. In addition, PCV-2 natural infection mainly
294 occurs during the nursery and growing periods, so, most of animals reach slaughterhouse
295 immunized and with low levels or no virus circulating [58]. On the other hand, PPV
296 vaccines are widely used in sows all over the world; considering the duration of PPV
297 maternally derived immunity [53], it was expected to have evidence of natural infection in
298 late finisher pigs. This aspect was confirmed with the present study.

299 Detection frequency of SIV RNA was very sporadic and the range of potential viral
300 contamination was variable. In IA, NC and Canada, 9%, 0% and 8% of samples yielded
301 positive results, respectively, and estimated amount of viable virus was <1.0 $\log_{10}$ $TCID_{50}$/g
302 SDPP. Similarly, the frequency of detection of SIV in Spain-C, Spain-NE, England, N-
303 Ireland and Brazil was 17%, 17%, 25%, 8% and 25%, respectively. However, when
304 present, had a very wide range of viral loads from 0.32 to 5.59 $Log_{10}$ $TCID_{50}$/g SDPP
305 (corresponding to -0.72 to 4.56 $Log_{10}$ $TCID_{50}$/g liquid raw plasma). It is speculated that

differences in stunning method, design of collection trough or slower line speed of abattoirs in Europe and Brazil compared to that in US and Canada may contribute to different levels of SIV contamination.

Estimated levels of infectious viruses in commercially collected pork plasma was significantly lower than viral levels at peak viremia of pigs [31, 46, 56, 59]. Commercially collected porcine plasma is harvested from animals that have been inspected and passed as fit for slaughter for human consumption, precluding collection of blood from clinically sick animals. Typically, market hogs have been vaccinated for many of the economically important diseases and have developed effective immunity [60, 61]. Therefore, data from this survey suggest that potential viral contamination in commercially produced SDPP is very low. Theoretical combined inactivation for all viruses analyzed in this study of multiple hurdles can inactivate more than 6 $\log_{10}$ $TCID_{50}$/g SDPP for spray drying and post drying storage and > 10 $\log_{10}$ $TCID_{50}$/g SDPP if UV-C if also included (Table 1).

In summary, the data from this survey allowed the calculation of potential viral contamination in commercially collected porcine plasma. Estimated level of viral contamination in commercially collected porcine plasma was very low ranging from <2 $\log_{10}$ $TCID_{50}$ for most viruses with infrequent SIV levels as high as 4.5 $\log_{10}$ $TCID_{50}$/g liquid plasma. Considering that the multiple hurdles in the manufacturing process (UV-C, spray drying and post drying storage) are theoretically capable of inactivating much higher levels of virus (11 to 20 $\log_{10}$ $TCID_{50}$), it is safe to assume that SDPP is a product devoid of infectious virus particles. These data suggest that the multiple hurdles in the manufacturing process of SDPP should be sufficient to inactivate much higher loads of viruses than the

328 potential viral contamination that can be detected in commercially collected porcine
329 plasma.

330

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335

## 336 Conflict of interest

337 The authors have read the journal's policy and the authors of this manuscript have the
338 following competing interests: EB, CR, and JPolo are employed by APC Europe, S.L.U.
339 Granollers, Spain and JC, LR and JPolo are employed by APC LLC, Ankeny, IA, USA. APC
340 Europe and APC LLC manufactures and sells spray-dried animal plasma; however, the
341 companies did not have any additional role in the study design, data collection and analysis,
342 decision to publish, or preparation of the manuscript. This does not alter the authors'
343 adherence to all PLOS ONE policies on sharing data and materials. JPujols, and JS declared
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## 346 References

1. Torrallardona D. Spray dried animal plasma as an alternative to antibiotics in weanling pigs. Asian-Australasian J Anim Sci. 2010; 23: 131–48. https://doi.org/10.5713/ajas.2010.70630
2. Remus A, Andretta I, Kipper M, Lehnen CR, Klein CC, Lovatto PA, et al. A meta-analytical study about the relation of blood plasma addition in diets for piglets in the post-weaning and productive performance variables. Livest Sci, 2013; 155: 294–300. https://doi.org/10.1016/j.livsci.2013.04.020
3. Pérez-Bosque A, Polo J, Torrallardona D. Spray dried plasma as an alternative to antibiotic in piglet feeds, mode of action and biosafety. Porcine Health Manag. 2016; 2:16. doi:10.1186/s40813-016-0034-1.
4. Campbell JM, Crenshaw JD, Gonzalez-Esquerra R, Polo J. Impact of spray-dried plasma on intestinal health and broiler performance. Microorganisms. 2019; 7: 219. doi:10.3390/microorganisms7080219.
5. Patterson AR, Madson DM, Opriessnig T. Efficacy of experimentally produced spray-dried plasma on infectivity of porcine circovirus type 21J Anim Sci. 2010; 88:4078–4085. doi:10.2527/jas.2009-2696.
6. Pasick J, Berhane Y, Ojkic D, Maxie G, Embuty-Hyatt C, Swekla K, et al. Investigation into the role of potentially contaminated feed as a source of the first-detected outbreaks of porcine epidemic diarrhea in Canada. Transbound Emerg Dis. 2014; 61: 397–410. https://doi.org/10.1111/tbed.12269
7. Aubry P, Thompson JL, Pasma T, Furness MC, Tataryn J. Weight of the evidence linking feed to an outbreak of porcine epidemic diarrhea in Canadian swine herds. J Swine Health & Prod. 2017; 25(2): 69–72. https://www.asi.k-state.edu/research-and-

371 extension/swine/Compressed%20Feed%20linked%20to%20PEDV%20outbreak%2
372 0in%20Canada.pdf
373 8. Messier S, Gagne-Fortin C, Crenshaw J. Dietary spray-dried porcine plasma
374 reduces mortality attributed to porcine circovirus associated disease syndrome.
375 Proc. Amer. Assoc. Swine Vet. 2007; p 147-150.
376 9. Pujols J, Segalés J, Polo J, Rodríguez C, Campbell J, Crenshaw J. Influence of
377 spray dried porcine plasma in starter diets associated with a conventional
378 vaccination program on wean to finish performance. Porcine Health Manag. 2016;
379 2:4. doi 10.1186/s40813-016-0021-6.
380 10. Dewey CE, Johnston WT, Gould L, Whiting TL. Postweaning mortality in
381 Manitoba swine. Can J Vet Res. 2006; 70:161–167. PMID: 16850937; PMCID:
382 PMC1477929.
383 11. Shen HG, Schalk S, Halbur PG, Campbell JM, Russell LE, Opriessnig T.
384 Commercially produced spray-dried porcine plasma contains increased
385 concentrations of porcine circovirus type 2 DNA but does not transmit porcine
386 circovirus type 2 when fed to naive pigs. J Anim Sci. 2011; 89:1930–1938.
387 doi:10.2527/jas.2010-3502.
388 12. Russell LE, Polo J, Meeker D. 2020. The Canadian 2014 porcine epidemic
389 diarrhoea virus outbreak: Important risk factors that were not considered in the
390 epidemiological investigation could change the conclusions. Transbound Emerg
391 Dis. 2020;67:1101–1112. doi: 10.1111/tbed.13496.
392 13. Polo J, Quigley JD, Russell LE, Campbell JM, Pujols J, Lukert PD. Efficacy of
393 spray-drying to reduce infectivity of pseudorabies and porcine reproductive and
394 respiratory syndrome (PRRS) viruses and seroconversion in pigs fed diets

containing spray-dried animal plasma. J Anim Sci. 2005; 83: 1933–1938. https//doi.org/10.2527/2005.8381933x

14. Pujols J, Rosell R, Russell L, Campbell J, Crenshaw J. Inactivation of swine vesicular disease virus in porcine plasma by spray-drying. Am Assoc Swine Vet. 2007; Perry, IA: p.281-284.
15. Gerber PF, Xiao C-T, Chen Q, Zhang J, Halbur PG, Opriessnig T. The spray-drying process is sufficient to inactivate infectious porcine epidemic diarrhea virus in plasma. Vet. Microbiol. 2014; 7;174(1-2):86-92. doi:10.1016/j.vetmic.2014.09.008.
16. Pujols J, Segalés J. Survivability of porcine epidemic diarrhea virus (PEDV) in bovine plasma submitted to spray drying processing and held at different time by temperature storage conditions. Vet Microbiol. 2014; 174: 427–432. doi: 10.1016/j.vetmic.2014.10.021.
17. Blázquez E, Rodríguez C, Ródenas J, Navarro N, Riquelme C, Rosell R, et al. Evaluation of the effectiveness of the SurePure Turbulator ultraviolet-C irradiation equipment on inactivation of different enveloped and non-enveloped viruses inoculated in commercially collected liquid animal plasma. PLoS One. 2019;14:e0212332. https://doi.org/10.1371/journal.pone.0212332.
18. Blázquez E, Rodríguez C, Ródenas J, Segalés J, Pujols J, Polo J. Biosafety steps in the manufacturing process of spray-dried plasma: a review with emphasis on the use of ultraviolet irradiation as a redundant biosafety procedure. Porcine Health Manag. 2020; 6:16. https://doi.org/10.1186/s40813-020-00155-1.
19. Fischer M, Pikalo J, Beer M, Blome S. Stability of African swine fever virus on contaminated spray dried porcine plasma. Transbound Emerg Dis. 2021;1-6. doi: 10.1111/tbed.14192.

20. WHO. Annex 4 Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products, vol.924: Geneva: World Health Organisation; 2004. p. 150–224.

21. Goodrich RP, Custer B, Keil S, Busch M. Defining "adequate" pathogen reduction performance for transfused blood components. Transfusion 2010; 50:1827-1837. doi: 10.1111/j.1537-2995.2010.02635.x

22. Reed MJ, Muench H. A simple method for estimating fifty percent end points. Am J Hyg. 1938; 27: 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408

23. Debouck P, Pensaert M. Experimental infection of pigs with a new porcine enteric coronavirus, CV 777. Am. J. Vet. Res. 1980; 41: 219–223. PMID: 6245603.

24. Baratelli M, Córdoba L, Pérez LJ, Maldonado J, Fraile L, Núñez JL, et al. Genetic characterization of influenza A viruses circulating in pigs and isolated in north-east Spain during the period 2006-2007. Res Vet Sci. 2014; 96: 380-388. doi: 10.1016/j.rvsc.2013.12.006.

25. Fort M, Sibila M, Nofrarías M, Pérez-Martín E, Olvera A, Mateu E, et al. Porcine circovirus type 2 (PCV2) Cap and Rep proteins are involved in the development of cell-mediated immunity upon PCV2 infection. Vet Immunol Immunopathol. 2010; 137: 226–234. doi: 10.1016/j.vetimm.2010.05.013.

26. Rodríguez-Arrioja GM, Segalés J, Calsamiglia M, Resendes AR, Balasch M, Plana-Duran J, et al. Dynamics of porcine circovirus type 2 infection in a herd of pigs with postweaning multisystemic wasting syndrome. Am J Vet Res. 2002; 63: 354–357. doi: 10.2460/ajvr.2002.63.354.

27. Parker J, Fowler N, Walmsley M-L, Schmidt T, Scharrer J, Kowaleski J, et al. Analytical sensitivity comparison between singleplex real-time PCR and a

multiplex PCR platform for detecting respiratory viruses. Plos One. 2015; 10(11):e0143164. doi: 10.1371/journal.pone.0143164.

28. Cochrane RA, Dritz SS, Woodworth JC, Huss AR, Stark CR, Hesse RA, et al. Evaluating Chemical Mitigation of Porcine Epidemic Diarrhea Virus (PEDV) in Swine Feed and Ingredients. Kansas Agricultural Experiment Station Research Reports 2015; Vol. 1: Iss. 7. http://dx.doi.org/10.4148/2378-5977.1110.
29. Dee S, Neill C, Clement T, Singrey A, Christopher-Hennings J, Nelson E. An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial. Porc Health Manag 2015; 1: 9. https://doi.org/10.1186/s40813-015-0003-0.
30. Houston E, Temeeyasen G, Piñeyro PE. Comprehensive review on immunopathogenesis, diagnostic and epidemiology of Senecavirus A. Virus Res. 2020; 286:198038. doi: 10.1016/j.virusres.2020.198038.
31. Joshi LR, Fernandes MHV, Clement T, Lawson S, Pillatzki A, Resende TP, et al. Pathogenesis of Senecavirus A infection in finishing pigs. J Gen Virol. 2016; 97: 3267–3279. doi: 10.1099/jgv.0.000631.
32. Zhang H, Chen P, Hao G, Liu W, Chen H, Qian P, et al. Comparison of the Pathogenicity of Two Different Branches of Senecavirus a Strain in China January 2020.Pathogens 2020; 9(1): 39. doi:10.3390/pathogens9010039.
33. Baker KL, Mowrer C, Canon A, Linhares DCL, Rademacher C, Karriker LA, et al. Systematic Epidemiological Investigations of Cases of Senecavirus A in US Swine Breeding Herds. Transbound Emerg Dis. 2017; 64:11–18. doi:10.1111/tbed.12598.
34. Leme RA, Alfieri AF, Alfieri AA. Update on Senecavirus Infection in Pigs. Viruses. 2017; 9:170. doi:10.3390/v9070170.

35. Schwegmann-Wessels C, Herrler G. Transmissible gastroenteritis virus infection: a vanishing specter. Dtsch Tierarztl Wochenschr. 2006; 113(4):157-159. PMID: 16716052.

36. Pensaert MB, Martelli P. Porcine epidemic diarrhea: a retrospect from Europe and matters of debate. Virus. Res. 2016; 226:1-6. doi: 10.1016/j.virusres.2016.05.030.

37. Chen F, Knutson TP, Rossow S, Saif LJ, Marthaler DG. Decline of transmissible gastroenteritis virus and its complex evolutionary relationship with porcine respiratory coronavirus in the United States. Scient Rep. 2019; 9:3953. https://doi.org/10.1038/s41598-019-40564-z.

38. Puente H, Argüello H, Mencía-Ares O, Gómez-García M, Rubio P, Carvajal A. Detection and genetic diversity of porcine cornavirus involved in diarrhea outbreaks in Spain. Front. Vet. Sci. 2021; 8: 651999. doi: 10.3389/fvets.2021.651999.

39. Ajayi T, Dara R, Misener M, Pasma T, Moser L, Poljak Z. Herd-level prevalence and incidence of porcine epidemic diarrhoea virus (PEDV) and porcine deltacoronavirus (PDCoV) in swine herds in Ontario, Canada. Transbound Emerg Dis. 2018; 65:1197-1207. doi: 10.1111/tbed.12858.

40. Crenshaw JD, Campbell JM, Polo J. Analysis of spray dried porcine plasma (SDPP) produced in Brazil and Western Canada confirm negative porcine epidemic diarrhea virus (PEDv) status of pigs in these regions. Proc. Allen D. Leman Swine Conf. 2014. Recent Research Reports, Univ. MN, St. Paul, MN. Sept. 13-16, 40:14.

41. Crenshaw J, Pujols J, Polo J, Campbell J, Rodríguez C, Navarro N, et al. Analysis of spray dried porcine plasma indicates absence of PRRSV infection in Brazilian pigs. 23rd IPVS Congress 2014. Cancun, México. June 8-11, 2014. p. 556. Poster 576.

491 42. Gava D, Caron L, Schaefer R, Santiago-Silva V, Weiblen R, Furtado-Flores E, et al.
492 A retrospective study of porcine reproductive and respiratory syndrome virus
493 infection in Brazilian pigs from 2008 to 2020. Transbound Emerg Dis. 2021; 00:1–
494 5. doi: 10.1111/tbed.14036.
495 43. OIE, World Organisation of Animal Health. World Animal Health Information
496 Database (WAHIS) Interface. Available from:
497 https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statuslist.
498 44. Rech RR, Gava D, Silva MC, Fernandes LT, Haach V, Ciacci-Zanbella JR, et al.
499 Porcine respiratory disease complex after the introduction of H1N1/2009 influenza
500 virus in Brazil. Zoonoses Public Health. 2018; 65(1):e155-e161.
501 doi:10.1111/zph.12424.
502 45. Zanella JRC, Morés N, de Barcellos DESN. Principais ameaças sanitárias
503 endêmicas da cadeia produtiva de suínos no Brasil. Pesq. agropec. bras., Brasília.
504 2016; 51 (5):443-453. doi: 10.1590/S0100-204X2016000500004.
505 46. Lyoo KS, Choi JY, Hahn TW, Park KT, Kim HK. Effect of vaccination with a
506 modified live porcine reproductive and respiratory syndrome virus vaccine on
507 growth performance in fattening pigs under field conditions. J Vet Med Sci. 2016;
508 78(9):1533-1536. doi: 10.1292/jvms.16-0137.
509 47. Alkhamis MA, Arruda AG, Vilalta C, Morrison RB, Perez AM. Surveillance of
510 porcine reproductive and respiratory syndrome virus in the United States using risk
511 mapping and species distribution modeling. Prev Vet Med. 2018;150, 135–142.
512 https://doi.org/10.1016/J.PREVETMED.2017.11.011
513 48. CSHIN quarterly producer report. Can Swine Health Intelligent Network. 2019.
514 Available from: https://www.cpc-

515 ccp.com/uploads/userfiles/files/CSHIN%202019%20Q3%20Producer%20Report_F
516 INAL%20EN.pdf
517 49. Machado G, Vilalta C, Recamonde-Mendoza M, Corzo C, Torremorell M, Pérez A,
518 et al. Identifying outbreaks of porcine epidemic diarrhea virus through animal
519 movements and spatial neighborhoods. Scient Rep. 2019; 9:457. doi:
520 10.1038/s41598-018-36934-8.
521 50. Saif L, Pensaert M, Sestak K, Yeo S, Jung K. Coronaviruses. In, Zimmerman,J.,
522 Karriker,L., Ramirez,A., Schwartz,K., and Stevenson,G. (eds), Diseases of Swine.
523 John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012:501–524.
524 51. Kong F, Xu Y, Ran W, Yin B, Feng L, Sun D - Cold Exposure-Induced Up-
525 Regulation of Hsp70 Positively Regulates PEDV mRNA Synthesis and Protein
526 Expression In Vitro. Pathogens. 2020; 9(4): 246.
527 https://doi.org/10.3390/pathogens9040246
528 52. Segalés J, Allan GM, Domingo M. Circoviruses. In: Zimmerman JJ, Karriker LA,
529 Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, editors. Diseases of swine. 11th
530 ed., Hoboken: John Wiley & Sons, Inc. 2019; 473-487.
531 doi:10.1002/9781119350927.
532 53. Truyen U, Streck AF. Parvoviruses. In: Zimmerman JJ, Karriker LA, Ramirez A,
533 Schwartz KJ, Stevenson GW, Zhang J, editors. Diseases of swine. 11th ed.,
534 Hoboken: John Wiley & Sons, Inc. 2019: 611–621. doi:10.1002/9781119350927.
535 54. Dvorak CMT, Yang Y, Haley C, Sharma N, Murtaugh MP. National reduction in
536 porcine circovirus type 2 prevalence following introduction of vaccination. Vet
537 Microb. 2016; 189: 86-90. doi: 10.1016/j.vetmic.2016.05.002.

538 55. Oliver-Ferrando S, Segalés J, López-Soria S, Callén A, Merdy O, Joisel F, et al.
539 Evaluation of natural porcine circovirus type 2 (PCV2) subclinical infection and
540 seroconversion dynamics in piglets vaccinated at different ages. M Vet Res. 2016;
541 47(1):121. https://doi.org/10.1186/s13567-016-0405-2
542 56. Opriessnig T, Gerber PF, Xiao C-T, Halbur PG, Matzinger SR, Meng X-J.
543 Commercial PCV2a-based vaccines are effective in protecting naturally PCV2b-
544 infected finisher pigs against experimental challenge with a 2012 mutant PCV2.
545 Vaccine. 2014; 32(34):4342-4348. doi: 10.1016/j.vaccine.2014.06.004.
546 57. Witvliet M, Holtslag H, Nell T, Segers R, Fachinger V. Efficacy and safety of a
547 combined porcine circovirus and Mycoplasma hyopneumoniae vaccine in finishing
548 pigs. Trials Vaccinol. 2015; 4:43-49. http://dx.doi.org/10.1016/j.trivac.2015.04.002.
549 58. Rose N, Opriessnig T, Grasland B, Jestin A. Epidemiology and transmission of
550 porcine circovirus type 2 (PCV2). Virus Res. 2012; 164(1-2):78-89. doi:
551 10.1016/j.virusres.2011.12.002.
552 59. Seo HW, Oh Y, Han K, Park C, Chae C. Reduction of porcine circovirus type 2
553 (PCV2) viremia by a reformulated inactivated chimeric PCV1-2 vaccine-induced
554 humoral and cellular immunity after experimental PCV2 challenge. BMC Vet Res.
555 2012; 8:194. https://doi.org/10.1186/1746-6148-8-194
556 60. PIC Gilt and Sow Management Guidelines. 2021. PIC-Gilt-Sow-Management-
557 Guidelines_05122%20(1).pdf
558 61. PIC Wean to Finish Guidelines. 2019. Wean-to-Finish-Manual-2019-
559 Final%20(1).pdf

62. Sampedro F, Snider T, Bueno I, Bergeron J, Urriola PE, Davies PR. Risk assessment of feed ingredients of porcine origin as vehicles for transmission of Porcine Epidemic Diarrhea Virus (PEDv). National Pork Board. 2015;1–117.

63. Blázquez E, Rodríguez C, Ródenas J, Rosell R, Segalés J, Pujols J, et al. Effect of spray-drying and ultraviolet C radiation as biosafety steps for CSFV and ASFV inactivation in porcine plasma. Plos One. 2021; 16(4): e0249935. https://doi.org/10.1371/journal.pone.0249935

**Table 1: Different inactivation steps involved in the manufacturing process of SDPP. Inactivation expressed as $Log_{10}$ $TCID_{50}$/g for viruses**

| Virus Type | | | Spray-Drying | UV-C* | Storage at 20°C for 14 d | Combined Theoretical Inactivation | References |
|---|---|---|---|---|---|---|---|
| RNA | Enveloped | Porcine reproductive and respiratory syndrome virus (PRRSV) | >4.0 | 12.9 ± 0.3 | >4.0 | >20.9 | [13, 17, 62] |
| | | Swine influenza virus (SIV) | 2.8** | 7.9 ± 0.2 | 3.2** | 13.9 | [17] |
| | | Porcine epidemic diarrhea virus (PEDV) | 5.1<br>4.2 | 6.6 ± 0.1 | 3.8 | 14.6-15.5 | [15-17] |
| | | Classical swine fever virus (CSFV) | 5.8 | 7.9 ± 0.2 | ND | >13.7 | [17, 63] |
| | Naked | Swine vesicular disease virus (SVDV) | 6.7 | 3.5 ± 0.07 | ND | >10.2 | [14, 17] |
| | | Senecavirus A (SVA) | ND | 4.0 ± 0.08 | >5.0** | >9.0 | [17] |
| DNA | Enveloped | Pseudorabies virus (PRV) | 5.3 | 8.1 ± 0.2 | ND | >13.4 | [13, 17] |
| | | African swine fever virus (ASFV) | 4.1 ± 0.2 | 6.8 ± 0.1 | >5.0 | >15.9 | [17, 19, 63] |
| | Naked | Porcine parvovirus (PPV) | 2.7** | 6.0 ± 0.1 | 3.1** | >11.8 | [17] |

Values with > results indicate the inactivated amount in the processed sample exceeded the amount inoculated in the initial sample before processing or storage.

*The UV log-kill estimated values were calculated commercial UV dosage (3251 J/L) by the estimated D-value from Blázquez et al., [17].

**University of Minnesota. Understanding the risk of virus transmission in spray dried porcine plasma – food safety assessment. 2020. Unpublished data

**Table 2. Ct values and estimated viral genome presence expressed in $Log_{10}$ genome equivalent copies (GEC) and $Log_{10}$ $TCID_{50}$/g SDPP in manufacturing plants located in different swine production areas around the world during the years 2018-2019. Values expressed as Average ± SD for only positive samples.**

| Plant | US-IA (n=11) | US-NC (n=12) | Canada (n=12) | Spain-NE (n=12) | Spain-C (n=12) | England (n=12) | NI (n=12) | Brazil (n=12) |
|---|---|---|---|---|---|---|---|---|
| PEDV | | | | | | | | |
| Ct | 33.46 ± 3.30 | 34.21 ± 2.06 | 33.94 | 35.09 ± 1.21 | 35.28 ± 1.39 | Neg | Neg | Neg |
| $Log_{10}$ GEC/g | 2.87 ± 0.89 | 2.67 ± 0.56 | 2.74 | 2.43 ± 0.33 | 2.38 ± 0.38 | | | |
| $Log_{10}$ $TCID_{50}$/g | 0.30 ± 0.92 | 0.10 ± 0.57 | 0.32 | 0.01 ± 0.33 | -0.05 ± 0.38 | | | |
| % Positive samples | 82 | 50 | 8 | 83 | 67 | 0 | 0 | 0 |
| PCV2 | | | | | | | | |
| Ct | 31.68 ± 0.56 | 30.98 ± 1.72 | 29.83 ± 0.91 | 30.39 ± 0.64 | 30.17 ± 0.96 | 30.63 ± 1.24 | 30.80 ± 0.67 | 31.02 ± 0.37 |
| $Log_{10}$ GEC/g | 5.29 ± 0.16 | 5.49 ± 0.50 | 5.67 ± 0.28 | 5.50 ± 0.20 | 5.57 ± 0.29 | 5.43 ± 0.38 | 5.38 ± 0.20 | 5.31 ± 0.11 |
| $Log_{10}$ $TCID_{50}$/g | 1.40 ± 0.15 | 1.59 ± 0.47 | 1.76 ± 0.26 | 1.60 ± 0.18 | 1.66 ± 0.27 | 1.53 ± 0.35 | 1.48 ± 0.19 | 1.42 ± 0.11 |
| % Positive samples | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| PPV | | | | | | | | |
| Ct | 30.44 ± 1.08 | 32.29 ± 2.44 | 30.64 ± 1.44 | 30.92 ± 3.25 | 30.58 ± 1.34 | 30.20 ± 1.08 | 28.42 ± 0.52 | 31.31 ± 1.02 |
| $Log_{10}$ GEC/g | 3.96 ± 0.25 | 3.53 ± 0.57 | 3.92 ± 0.34 | 3.85 ± 0.77 | 3.93 ± 0.32 | 4.02 ± 0.25 | 4.44 ± 0.12 | 3.76 ± 0.24 |
| $Log_{10}$ $TCID_{50}$/g | 2.81 ± 0.27 | 2.35 ± 0.61 | 2.79 ± 0.36 | 2.72 ± 0.81 | 2.81 ± 0.33 | 2.90 ± 0.27 | 3.34 ± 0.13 | 2.62 ± 0.25 |
| % Positive samples | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| SIV | | | | | | | | |
| Ct | 37.55 | Neg | 35.42 | 23.17 ± 3.83 | 19.56 ± 0.28 | 23.62 ± 10.58 | 20.93 | 27.60 ± 9.53 |
| $Log_{10}$ GEC/g | | | | | | | | |
| $Log_{10}$ $TCID_{50}$/g | -1.33 | | 0.44 | 3.93 ± 1.09 | 4.96 ± 0.08 | 3.80 ± 3.02 | 4.57 | 2.67 ± 2.72 |
| % Positive samples | 9 | 0 | 8 | 17 | 17 | 25 | 8 | 25 |
| PRRS-US | | | | | | | | |
| Ct | 33.36 ± 1.64 | 34.29 ± 1.26 | 34.14 ± 2.24 | Neg | 36.08 | Neg | Neg | Neg |
| $Log_{10}$ GEC/g | 2.40 ± 0.48 | 2.13 ± 0.37 | 2.17 ± 0.66 | | 1.62 | | | |
| $Log_{10}$ $TCID_{50}$/g | -1.25 ± 0.51 | -1.52 ± 0.39 | -1.49 ± 0.69 | | -2.09 | | | |
| % Positive samples | 100 | 17 | 50 | 0 | 8 | 0 | 0 | 0 |
| PRRS-EU | | | | | | | | |
| Ct | 35.70 | Neg | Neg | 34.51 ± 0.88 | 33.88 ± 1.60 | 33.64 ± 1.44 | 33.90 ± 1.04 | Neg |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $Log_{10}$ GEC/g | 2.08 | | | 2.42 ± 0.25 | 2.60 ± 0.46 | 2.67 ± 0.41 | 2.59 ± 0.30 | |
| $Log_{10}$ $TCID_{50}$/g | -0.29 | | | 0.03 ± 0.24 | 0.20 ± 0.43 | 0.26 ± 0.39 | 0.19 ± 0.28 | |
| % Positive samples | 9 | 0 | 0 | 33 | 58 | 50 | 83 | 0 |

580

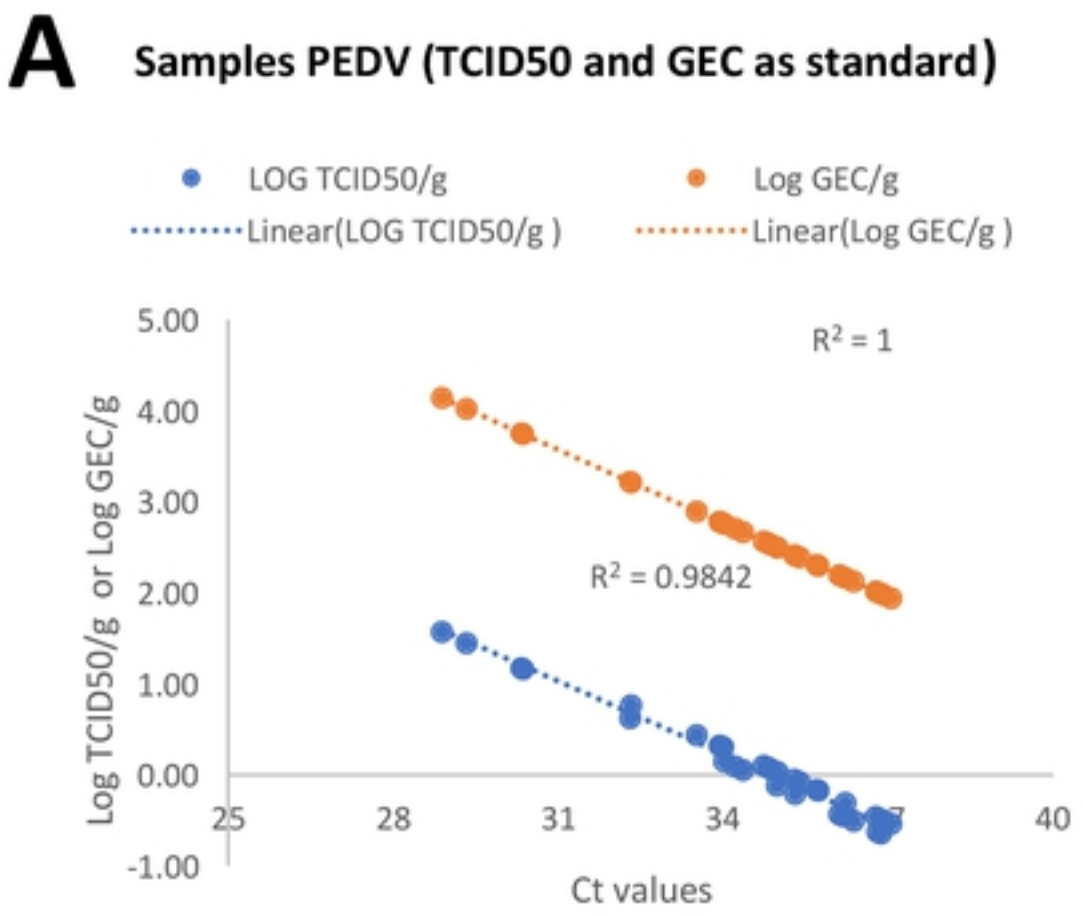
**Table 3. Estimated quantification of different viruses' genomes expressed in $Log_{10}$ $TCID_{50}$/g ± SD (percentage of positive samples) in unprocessed raw liquid plasma from PCR or RT-PCR analyses of SDPP samples collected at different plants.**

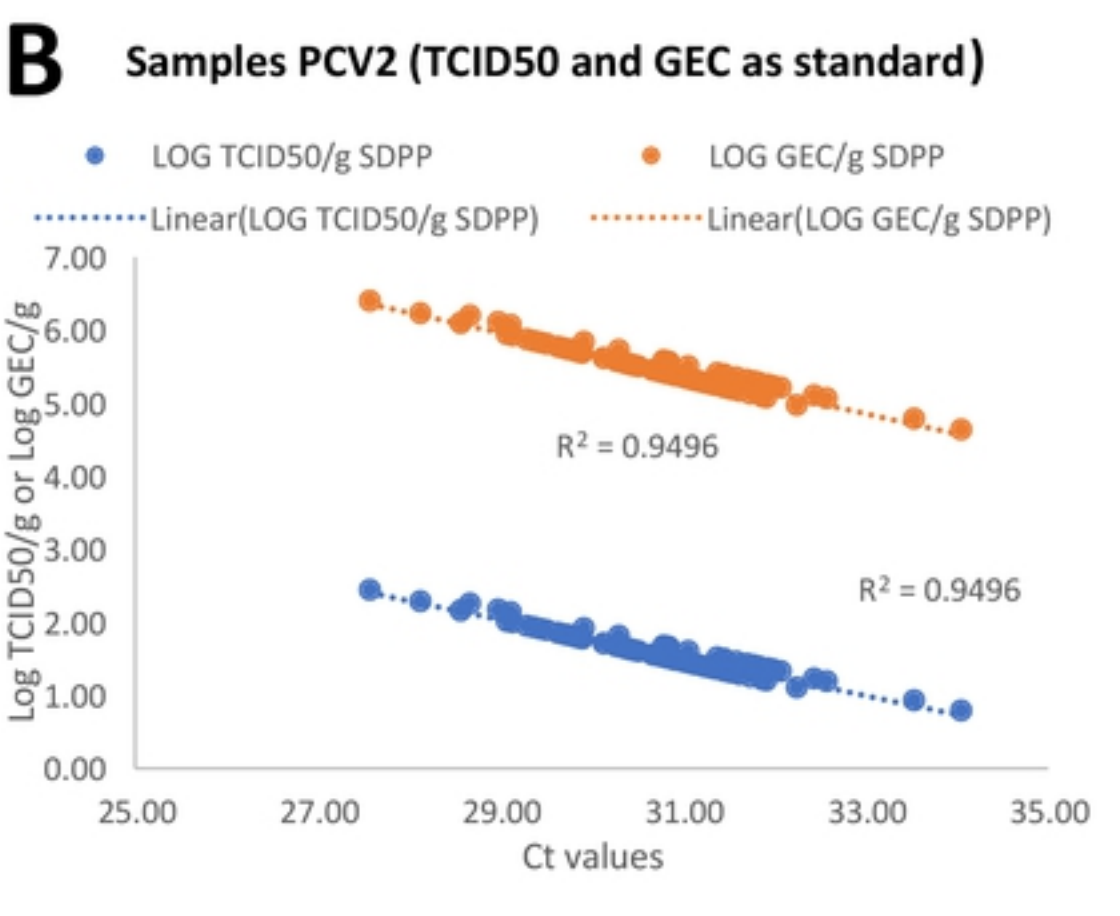
| Plant | **PEDV** | **PCV2** | **PPV** | **SIV** | **PRRS- US** | **PRRS-EU** |
|---|---|---|---|---|---|---|
| US-IA | -0.82 ± 0.92 | 0.28 ± 0.15 | 1.69 ± 0.27 | -2.45 | -2.37 ± 0.51 | -1.42 |
| US-NC | -0.94 ± 0.57 | 0.55 ± 0.47 | 1.31 ± 0.61 | Neg | 2.58 ± 0.39 | Neg |
| Canada | -0.80 | 0.64 ± 0.26 | 1.67 ± 0.36 | -0.69 | -2.48 ± 0.65 | Neg |
| Spain-NE | -1.03 ± 0.31 | 0.56 ± 0.18 | 1.68 ± 0.81 | 2.89 ± 1.09 | Neg | -1.03 ± 0.25 |
| Spain-C | -1.17 ± 0.38 | 0.54 ± 0.27 | 1.68 ± 0.33 | 3.84 ± 0.08 | -3.21 | -0.93 ± 0.43 |
| England | Neg | 0.49 ± 0.35 | 1.86 ± 0.27 | 2.76 ± 3.02 | Neg | -0.78 ± 0.39 |
| Northern Ireland | Neg | 0.36 ± 0.19 | 2.22 ± 0.13 | 3.45 | Neg | -0.93 ± 0.28 |
| Brazil | Neg | 0.30 ± 0.11 | 1.50 ± 0.25 | 1.56 ± 2.70 | Neg | Neg |
| | | | | | | |
| Range | -1.76 – -0.57 | -0.28 – 1.37 | -0.15 – -2.55 | -2.45 – 4.56 | -3.23 – -1.52 | -1.47 – -0.18 |

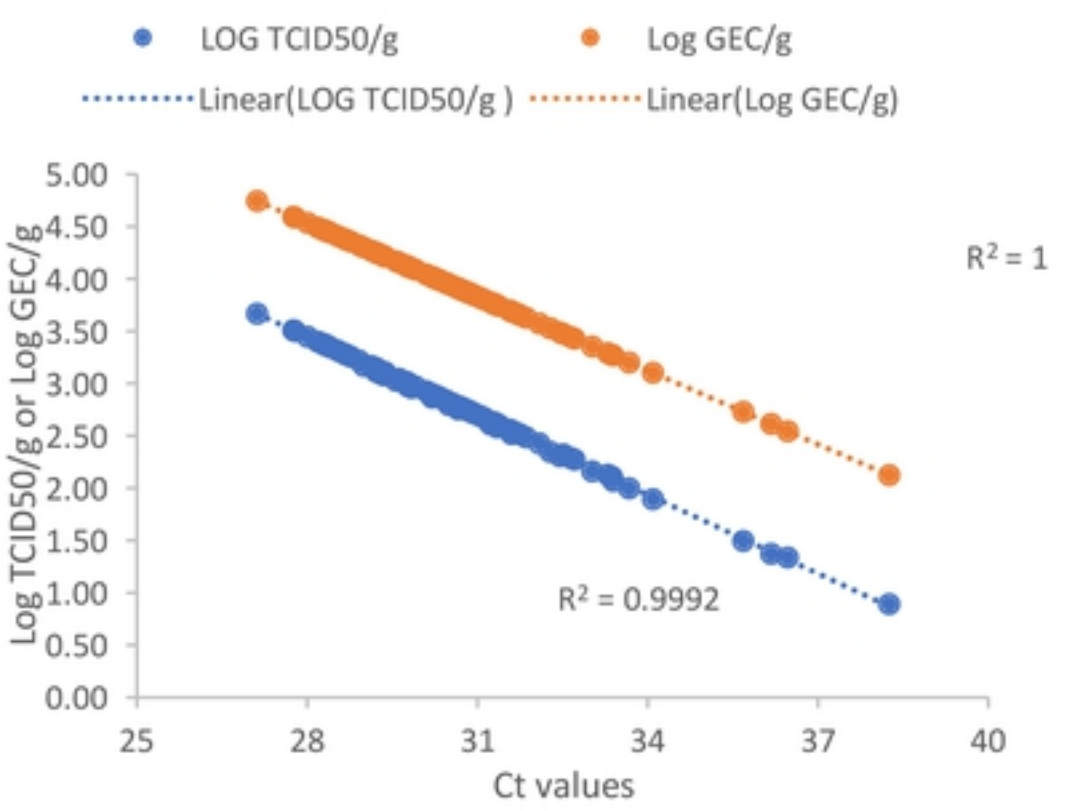
**Figure 1.** Regression curves between Ct values and tissue culture infections dose 50 ($TCID_{50}$/g) or Genome equivalent copies (GEC)/g of spray-dried porcine plasma (SDPP).

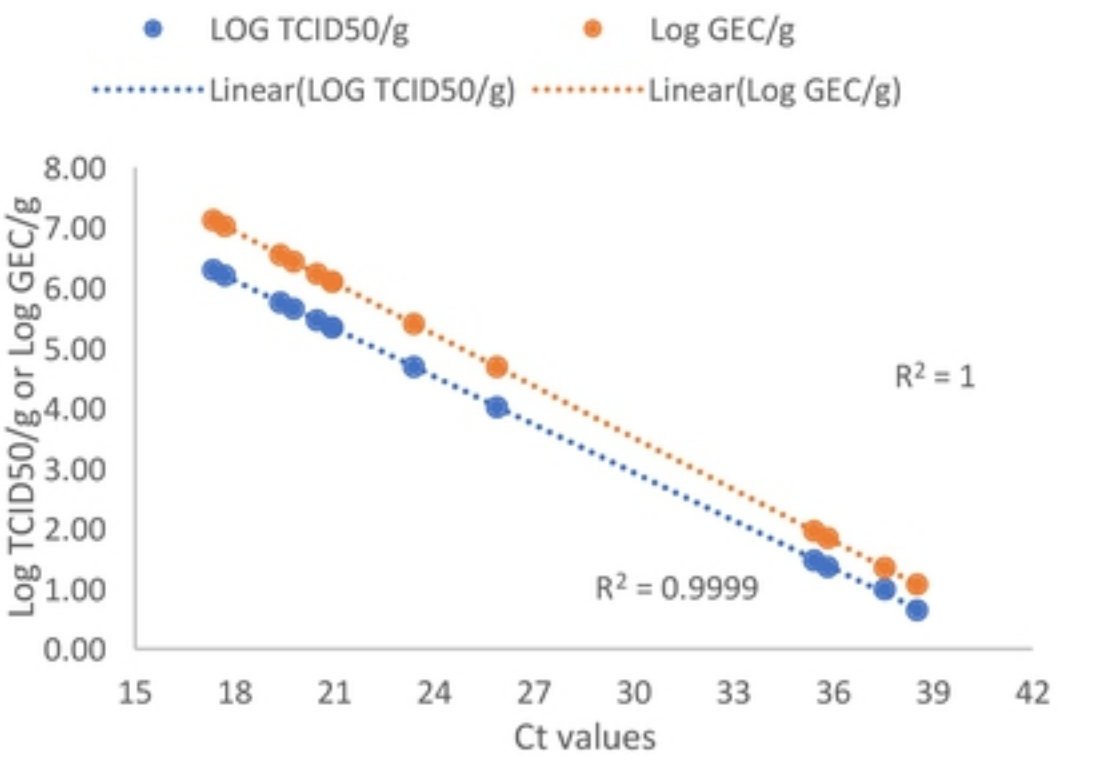
Values expressed in $Log_{10}$ $TCID_{50}$/g SDPP or $Log_{10}$ GEC/g SDPP. Each box includes the spot values of the SDPP samples analyzed and the regression equation between CT and $TCID_{50}$/g or GEC/g SDP and the $r^2$ value.

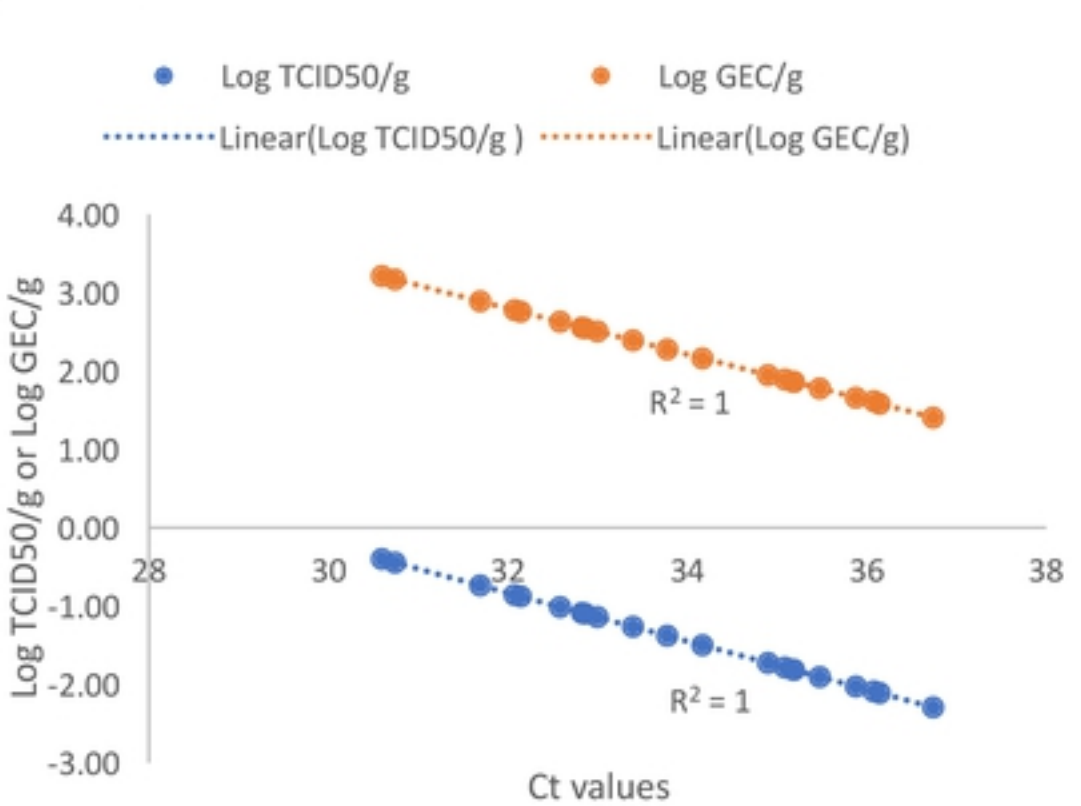
A.Regression curves for *porcine epidemic diarrhea virus* (PEDV); B. Regression curves for *porcine circovirus type-2* (PCV2); C. Regression curves for *porcine parvovirus* (PPV); D. Regression curves for *swine influenza virus* (SVI) H1N1; E. Regression curves for *porcine reproductive and respiratory syndrome virus* (PRRSV) US strain; F. Regression curves for PRRSV EU strain.

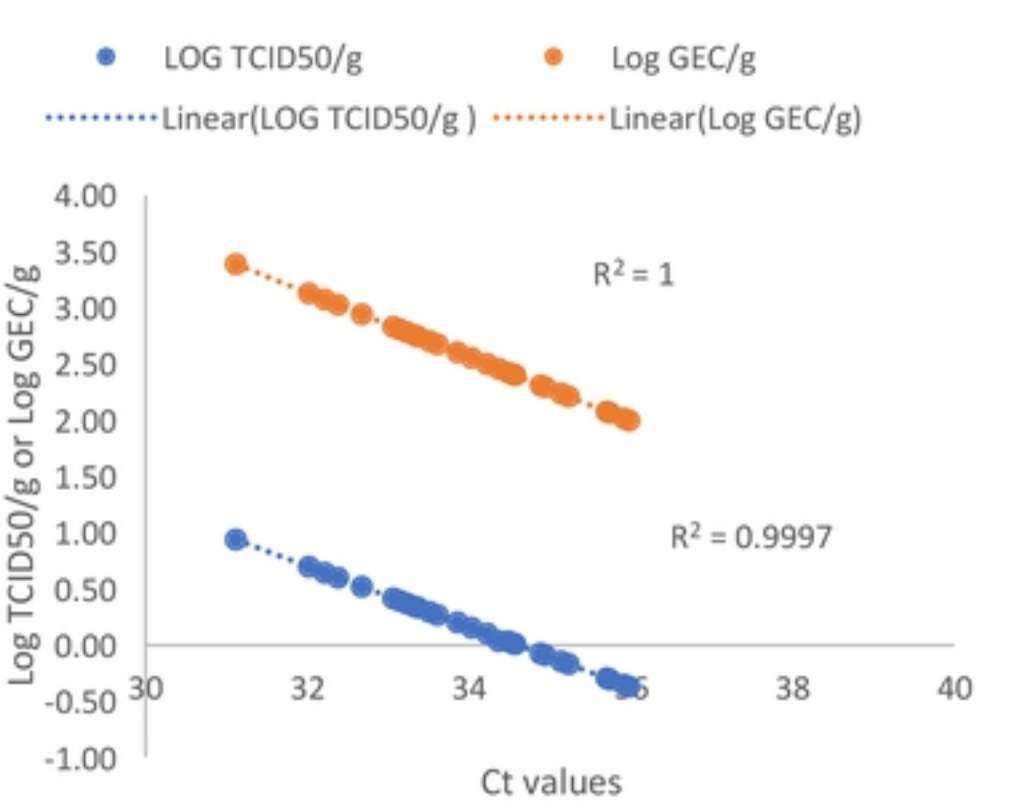



Figure