# Survivability of porcine epidemic diarrhea virus (PEDV) in bovine plasma submitted to spray drying processing and held at different time by temperature storage conditions

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## ARTICLE INFO

## ABSTRACT

Bovine plasma was inoculated with porcine epidemic diarrhea virus (PEDV) at an average final titer of 4.2 $\log10$ $TCID_{50}$/mL to determine the effect of spray drying on viral inactivation. Using a laboratory scale drier, inoculated plasma was spray dried at 200 °C inlet temperature and either 70 or 80 °C throughout substance. Both liquid and dried samples were subjected to three passages on VERO cell monolayers to determine PEDV infectivity. Results indicated liquid samples contained infective virus, but none of the spray dried samples were infectious. Also, survivability of PEDV inoculated on spray dried bovine plasma (SDBP) and stored at 4, 12 or 22 °C was determined for 7, 14 and 21 days. Commercial SDBP powder was inoculated with PEDV to an average final titer of 2.8 $\log10$ $TCID_{50}$/g. Five samples per time and temperature conditions were subjected to three passages on VERO cell monolayers to determine PEDV infectivity. The virus was non-infectious for all samples stored at 22 °C at 7, 14 and 21 days. PEDV was infective in 1 out of 5 samples stored at 12 °C at 7 days, but none of the samples stored for 14 and 21 days were infectious in cell culture. For samples stored at 4 °C, 4 out of 5 samples were infectious at 7 days, 1 out of 5 samples were infectious at 14 days, but none were infectious at 21 days. In summary, PEDV was not infectious on cell culture within 7 days when stored at room temperature and within 21 days when stored at refrigerated temperature.

## 1. Introduction

Porcine epidemic diarrhea (PED) virus (PEDV) is an RNA enveloped virus ranging in diameter from 90 to 190 nm, which is taxonomically classified into the Coronaviridae family (Song and Park, 2012). PEDV is antigenically unrelated to other porcine coronaviruses like gastroenteritis transmissible virus and hemaglutinating encephalomyelitis virus (Kusanagi et al., 1992). PEDV is highly infectious and transmission is primarily through pig-to-pig contact or indirect contact with fecal material from infected pigs.

PED was linked to diarrhea in nursery and fattening pigs in Europe in 1971, and PEDV was initially recognized in 1978 in Belgium (Pensaert and Debouck, 1978). PED has also been documented in South-East Asia since the 1980s (Song and Park, 2012). However, variant strains of PEDV causing severe outbreaks of disease in suckling pigs with high morbidity and mortality appeared in 2010 in China (Li et al., 2012). Furthermore, and since April 2013, very similar PEDV strains to the Chinese variants have been

detected in North America resulting in significant pig mortality and economic loss (Huang et al., 2013). Phylogenetic analyses suggested that the PEDV in the USA likely originated from China (Huang et al., 2013). Taking into account that the exact source of origin of the US strains has not been identified, the potential continued spread of the virus worldwide is a critical concern. Under this scenario, feed or feed ingredients, including spray-dried porcine plasma (SDPP), have recently been suggested as potential sources for transmission of PEDV (Pasick et al., 2014).

Spray-dried plasma (SDP) proteins are used extensively in nursery pig feed to enhance feed intake, growth, and feed efficiency during the post-weaning period (Coffey and Cromwell, 2001; Van Dijk et al., 2001) and represents an excellent alternative to antibiotics (Torrallardona, 2010). The spray drying process used to manufacture SDP involves sudden changes in temperature and pressure that causes rapid evaporation of water which is detrimental to a variety of pathogens including bacteria and viruses (Polo et al., 2005, 2013). A series of studies have demonstrated that manufacturing conditions to produce commercial spray-dried porcine plasma (SDPP) inactivate several viruses of importance to the swine industry such as porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV) (Polo et al., 2005) or swine vesicular disease virus (SVD) (Pujols et al., 2007). In addition, several studies have reported that orally consumed commercial SDPP does not transmit either porcine parvovirus (PPV) or porcine circovirus type 2 (PCV2) (Polo et al., 2005; Pujols et al., 2008, 2011; Shen et al., 2011), which are two of the most heat and chemically resistant swine viruses.

The first objective of this study was to determine the survival of PEDV inoculated in liquid bovine plasma that was then submitted to two different spray drying conditions (70 and 80 °C throughout its substance). A second objective was to determine survival time of PEDV inoculated on spray-dried bovine plasma when stored under different temperatures (4, 12 and 22 °C) for different periods of time (0, 7, 14 and 21 days).

## 2. Materials and methods

### 2.1. Virus preparation and detection

The CV777 strain (Pensaert and Debouck, 1978; Debouck and Pensaert, 1980), prototype PEDV strain from Belgium, belongs to group G1-1 and is similar to isolates Br-1/87 (Britain), JS-2004 (China), P-5V (Japan) and KPED-9, SM98-1 and DR-13 (South Korea) (Park et al., 2007). This strain of PEDV is well known to propagate on VERO cells and was selected as a model swine coronavirus to study inactivation of PEDV by spray drying. PEDV was propagated in VERO cell line, grown in Eagle's Minimum Essential Medium (MEM), supplemented with 0.3% tryptose phosphate broth (Sigma), 10% fetal bovine serum and an antibiotic combination (penicillin 100 IU/mL, streptomycin 100 μg/mL). Virus stock was produced in VERO cells seeded in 175 cm$^2$ flask to confluent monolayers, cells were rinsed 3 times with phosphate buffered saline and inoculated with 10 ml of PEDV 3.5 log10 TCID$_{50}$/mL (MOI

of 0.002) to each bottle. The virus-cells mixture was incubated 1 h at 37 °C, and then the inoculum was replaced with post-infection media (PM), which consisted of MEM supplemented with 0.3% tryptose phosphate broth (Sigma), 12.5 μg/mL trypsin (Sigma) and the above mentioned antibiotic combination (Hofmann and Wyler, 1989). Cultures were checked at 18 and 24 h after infection to assess syncytia formation and the evolution of cytopathogenic effect (CPE) until reaching nearly 100% CPE. Liquid supernatants and scraped cells were collected. The pooled suspensions were centrifuged at 1000 g for 20 min at 4 °C and supernatants were stored at 4 °C. The resulting pellet was suspended in PM and subjected to freeze-thaw, centrifuged and supernatants were harvested and added to bulk supernatants. Stock virus was stored at −80 °C or used immediately after its production (Hofmann and Wyler, 1989). A PEDV virus stock was produced to a virus titer ranging between 4.0 log10 and 5.0 log10 TCID$_{50}$/mL (Hofmann and Wyler, 1989; Song et al., 2003; Oh et al., 2005).

Total PEDV RNA genome copies of virus production was estimated by quantitative reverse-transcriptase PCR (qRT-PCR) with a swine enteric panel assay for PEDV and other enteric viruses (Thermo Fisher Scientific Inc., Waltham, MA). Correlation between PEDV loads by TCID50/mL on cell culture and Ct values by qRT-PCR was calculated. For this, 10-fold dilutions of $4.6 \pm 0.2$ log10 TCID$_{50}$ of strain PEDV CV777 were extracted two times and amplified 3 times each one.

### 2.2. Experiment 1: inactivation of PEDV by the spray drying process

#### 2.2.1. Study design

The reason for using bovine plasma was mainly to avoid the presence of potential antibodies against PEDV European strains or cross-reaction with antibodies against other porcine coronavirus that may be present in swine plasma. Bovine plasma was collected from an EU inspected slaughter facility. Animals were inspected and approved for slaughter for human consumption. Blood was collected into stainless steel pans containing sodium phosphate as anticoagulant. Blood was transported refrigerated to the laboratory and plasma was separated by centrifugation and three 0.135-L bottles were prepared and frozen at −20 °C. A 10-mL sample of each of the 0.135-L batches was stored at −80 °C and used as controls to determine absence of virus neutralization antibodies and viral contamination.

Each batch of plasma was inoculated with stock PEDV at 1:1 dilution. PEDV was added to each 0.125 L of bovine plasma to get an approximate virus titer of 4.0 log10–4.5 log10 TCID$_{50}$/mL.

Each of the three 0.25-L batches was sampled in three 5 mL tubes just after the virus inoculation and immediately frozen at −80 °C. The three 0.235-L batches were dried using a Büchi 190 Mini Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland). All the bottles were dried with an inlet temperature of 200 ± 5 °C and outlet temperature of 80 ± 1 °C. Before spray-drying plasma and virus mixture, the spray-drier was drying control bovine plasma at the target temperature for 45 min to ensure that the system was

processing at the target parameters when the virus inoculated plasma was dried. The spray drying parameters used were similar to those previously established (Polo et al., 2005). Airflow (at 20 °C) through the column was set at 45 m$^3$ h$^{-1}$ and the suspension flow to the nozzle was set at 0.2 m$^3$ h$^{-1}$. The airflow through the feeding nozzle was set at 0.7 m$^3$ h$^{-1}$ (at 20 °C). Estimated dwell time was 0.41 s.

The dwell time of industrial size driers used in manufacturing plants to produce commercial SDP ranges between 20 and 90 s, depending on size of the spray drier and configuration (fluid bed, etc.), whereas the dwell time of the lab drier was <1 s. In an attempt to more closely simulate industrial size driers and conditions, immediately after the spray-drying process, 6 sub-samples (0.5 g each) of SDP were placed in 0.5 cm (inner diameter) glass tubes and held at 89–91 °C in a water bath for a dwell time of 30 s or 60 s to achieve an interior plasma powder temperature of 70.4 °C and 80.7 °C, respectively. The temperature was determined with thermocouple probe (Crison Instruments SA, Alella, Spain) inserted in the glass tube containing the SDBP. Dried samples were immediately frozen in dry ice and stored at −80 °C prior to analysis.

### 2.2.2. PEDV infectivity in cell culture

Liquid and dried frozen samples were analyzed for infectivity in VERO cell cultures, using the microtiter assay procedure. Dried samples were reconstituted by adding 0.5 g of plasma to 5.5 mL of distilled water to each sample. To determine that no minute quantities of virus had survived, 5 mL of each of the reconstituted spray-dried samples were inoculated on a 75 cm$^2$ flask of VERO cell monolayers. After 1 h of incubation at 37 °C the inoculum was discarded and cultures were rinsed 3 times with PBS and then PM media was added. After four days of incubation at 37 °C and 5% CO$_2$, cell cultures were frozen-thawed, harvested, gently vortex to full homogenization and 5 ml supernatants seeded onto new 75 cm$^2$ bottles with VERO cell monolayers. Three serial passages were made. If CPE was observed, then the initial sample was titrated on VERO monolayers grown in 96-well tissue culture plates.

### 2.2.3. Virus neutralization test

A virus neutralization (VN) test was performed on the three liquid samples to verify that the bovine plasma used had no neutralizing antibodies against PEDV. Briefly, 2 log dilutions of bovine plasma were incubated 1 h at 37 °C with 200 TCID$_{50}$/mL of PEDV. Then 0.1 mL of this mixture was added to a VERO cells monolayer grown in 96-well plates that previously had been rinsed 3 times with PBS, and were incubated for 1 h at 37 °C. After adsorption, inoculum was removed and plates were rinsed 3 times with PBS. PM was added and plates were incubated for 3 days at 37 °C. Virus neutralization was expressed as the reciprocal of highest serum dilution that abrogates the CPE (Oh et al., 2005).

### 2.3. Experiment 2: survival of PEDV inoculated on spray dried bovine plasma stored over time at different temperatures

Spray dried bovine plasma (SDBP) was selected from commercial production for use in the experiment. A 10-g sample of the SDBP was stored at −80 °C and used as a control to determine absence of PEDV neutralization antibodies and PEDV contamination. Sixty grams of SDBP contained in a large Petri dish were inoculated with 9.0 mL of PEDV cell culture by spraying the liquid virus culture onto the SDBP using a spray bottle. The Petri dish was sealed with plastic paraffin film and shaken vigorously for 15 min to ensure a good homogenization of the virus with the SDBP.

One gram of inoculated SDBP was added to 50 different glass tubes (0.5 cm inner diameter). One set of 15 tubes was stored at 4 ± 2 °C in the lab refrigerator, another set of 15 tubes was stored at 12 ± 2 °C in a calibrated water bath and the last set of 15 tubes was stored at room lab temperature of 22 ± 2 °C. For each temperature storage condition, 5 tubes were selected at 7, 14 and 21 days and stored at −80 °C until their analysis. At the time of analysis, the −80 °C stored samples (the initial samples and the samples stored at different time by temperature conditions) were analyzed to detect the survival of PEDV in cell culture. Each dried sample was reconstituted by adding 1 g of SDBP to 10 mL of distilled water. To determine that no minute quantities of virus survived, 5 mL of each of the reconstituted SDBP samples were inoculated on a 75 cm$^2$ flask of VERO cell monolayers and, after 1 h incubation at 37 °C, the inoculum was discarded and cultures were rinsed 3 times with PBS and then PM media was added. After four days, cell cultures were thawed, harvested, clarified by centrifugation and passaged to new VERO cell cultures. Three serial passages were made. If CPE was observed, then virus was titrated on VERO cell monolayers grown in 96-well tissue culture plates.

A tissue culture VN test was performed on the control SDBP used to ensure lack of neutralizing antibodies against PEDV in the original sample. Briefly, 2 log dilutions of SDBP re-suspended in distilled water (0.5 g SDBP to 5 mL distilled water) were incubated 1 h at 37 °C with 200 TCID$_{50}$/mL of PEDV. Then, the same procedure explained before for liquid bovine plasma was followed.

### 2.4. Statistical analyses

Statistical analysis were performed on qRT-PCR results by GLM procedure and Turkey-Kramer multiple comparison test (NCSS 2004, Statistical Systems, NCSS LLC, Kaysville, UT).

## 3. Results

### 3.1. Correlation between PEDV titration and quantification by qRT-PCR

Correlation between PEDV titers expressed as TCID$_{50}$/mL and PEDV loads expressed in qRT-PCR Ct values are displayed in Fig. 1. Under the range of detection on cell culture, the qRT-PCR results displayed an increased range of standard deviation, which was attributed to non-encapsidated virus material, still present at higher dilutions. The regression results showed a close correlation ($r = 0.9745$; $y = -3.30x + 28.60$).
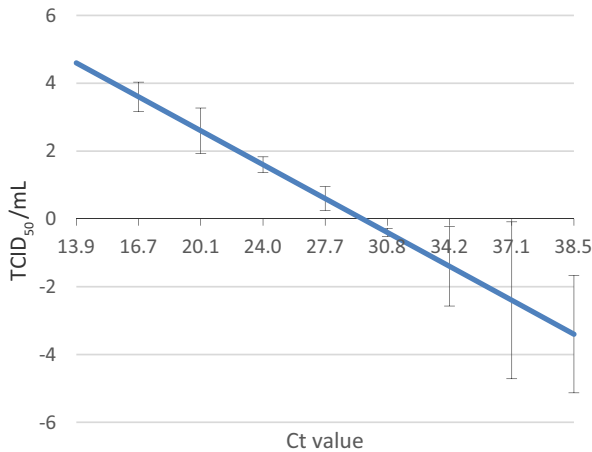
**Fig. 1.** Correlation between PEDV $TCID_{50}$/mL titers and quantitative reverse transcriptase PCR Ct values. Values are expressed as mean and standard deviation.

### 3.2. Experiment 1: inactivation of PEDV by the spray drying process

The non-inoculated liquid bovine plasma stored at $-80\,°C$ was determined negative for presence of PEDV and negative for neutralizing antibodies against the virus. Total concentration of PEDV in the pellet used to inoculate the liquid bovine plasma was determined to be 4.6 log10 $TCID_{50}$/mL (Ct = 12.1 ± 0.2 by means of qRT-PCR). Results of the liquid and the SDBP samples are presented in Table 1.

Analyses of the initial 3 batches of inoculated liquid bovine plasma stored at $-80\,°C$ gave an average titer of 4.2 ± 0.2 log10 $TCID_{50}$/mL (calculated for dry product: 5.15 ± 0.2 log10 $TCID_{50}$/g).

The samples processed at both temperatures (70 and $80\,°C$) throughout its substance were negative for PEDV infectivity on cell culture. The calculated inactivation rate at these temperatures was at least greater than the initial titer of 5.15 log10 TCID $TCID_{50}$/g. The qRT-PCR Ct results for the virus inoculated plasma were 13.9 ± 0.3, and for the spray dried plasma at 70 and $80\,°C$ throughout its substance were 23.3 ± 0.6 and 23.8 ± 0.8, respectively.

### 3.3. Experiment 2: survival of PEDV inoculated on spray dried bovine plasma stored over time at different temperatures

The non-inoculated SDBP stored at $-80\,°C$ was determined negative for PEDV in VERO cell culture and qRT-PCR. Total concentration of PEDV in the pellet used for SDBP

inoculation was titrated to be 4.6 log10 $TCID_{50}$/mL (Ct = 12.1 ± 0.2). The expected titer after virus inoculation was 3.8 log10 $TCID_{50}$/g.

Results of the SDBP PEDV inoculated samples are presented in Table 2. Analyses of the initial 5 contaminated SDBP samples that were immediately stored at $-80\,°C$ averaged 2.8 log10 $TCID_{50}$/g (average Ct = 22.0 ± 0.8). For samples stored at $4 ± 2\,°C$, 4 out of the 5 samples contained infectious PEDV at day 7 of storage, but at day 14 only 1 out of the 5 samples contained infectious virus and none of the samples contained infectious PEDV by day 21 of storage. The Ct value for samples stored at $4 ± 2\,°C$ averaged 21.6 ± 0.7. For samples stored at $12 ± 2\,°C$, only 1 out of the 5 samples stored for 7 days contained infectious virus and by days 14 and 21 none of the samples retained infectivity. The Ct value for samples stored at $12 ± 2\,°C$ averaged 22.2 ± 1.1. For samples stored at room temperature ($22 ± 2\,°C$), none of them remained infectious at 7, 14 and 21 days of storage. The Ct value for samples stored at $22\,°C$ averaged 21.1 ± 0.6.

## 4. Discussion

The primary route of infection of PEDV is direct contact with infected pigs or from the manure of infected pigs (OIE, 2014). Other routes of infection responsible for spreading the virus have not been clearly identified, but transport vehicles for swine and feed or feed ingredients have been shown to contain PEDV genome by RT-PCR (Dee et al., 2014; OIE, 2014).

The role of feed or feed ingredients in the spread of PEDV was suggested after the first report of PED in Ontario (Canada). The subsequent investigation conducted by the Canadian Food Inspection Agency (CFIA) concluded that nursery feed containing SDPP might have been a source of PEDV transmission. Although the CFIA reported that infective virus was detected in samples of SDPP, infective virus could not be detected in the feed containing the corresponding SDPP (Pasick et al., 2014). In spite of this conflicting data, the opinion that SDPP may contain infective PEDV as a reason to explain the spread of PEDV across North America has been adopted by some swine industry professionals.

The results obtained in the present study indicated that PEDV inoculated in bovine plasma was apparently inactivated when spray-dried at both simulated commercial processing conditions of 70 and $80\,°C$ throughout its substance. These results are in agreement with previous publications indicating that PEDV is a low thermal stable virus (Song and Park, 2012). In addition, these results agree with previous reports with different enveloped

**Table 1**

Porcine epidemic diarrhea virus (PEDV) titer of inoculated liquid and spray-dried bovine plasma[a].

| Spray drying condition | Inoculated liquid plasma PEDV $TCID_{50}$/mL | Spray-dried plasma PEDV $TCID_{50}$/g |
|---|---|---|
| 70 °C throughout substance | 4.2 ± 0.2 log10 (Ct: 13.9 ± 0.3) | Not detected (Ct: 23.3 ± 0.6)[b,c] |
| 80 °C throughout substance | 4.2 ± 0.2 log10 (Ct: 13.9 ± 0.3) | Not detected (Ct: 23.8 ± 0.8) |

[a] Data represents the mean PED virus isolation results of triplicate analysis of three lots per liquid and spray dried condition that had been subjected to three serial passages on VERO cell monolayers.

[b] The spray dryer concentrated liquid plasma 10 times; the expected virus titer in the dry product was 5.15 ± 0.2 log10 $TCID_{50}$/g.

[c] The theoretical limit of detection of the method used was estimated to be able to detect as low as 0.7 viral particles/g.

**Table 2**

Porcine epidemic diarrhea virus (PEDV) titer of inoculated spray-dried bovine plasma samples held at various time by temperature conditions[a].

| Temp. | Time | Ct values[b] | PEDV titer ($TCID_{50}$/g) per sample number[c] | | | | |
|---|---|---|---|---|---|---|---|
| (°C) | (d) | (mean ± SD) | 1 | 2 | 3 | 4 | 5 |
| −80 | 0 | 22.0 ± 0.8 | Average 2.8 log10 | | | | |
| 4 ± 2 | 7 | 21.6 ± 0.7[a] | 0.8 log10 | 0.8 log10 | 0.8 log10 | 0.93 log10 | Neg |
| 4 ± 2 | 14 | 30.9 ± 0.6[b] | Neg | Neg | Neg | 0.8 log10 | Neg |
| 4 ± 2 | 21 | 31.5 ± 0.3[c] | Neg | Neg | Neg | Neg | Neg |
| 12 ± 2 | 7 | 22.2 ± 1.1[a] | Neg | Neg | Neg | Neg | 0.8 log10 |
| 12 ± 2 | 14 | 24.3 ± 1.0[b] | Neg | Neg | Neg | Neg | Neg |
| 12 ± 2 | 21 | 31.1 ± 0.3[c] | Neg | Neg | Neg | Neg | Neg |
| 22 ± 2 | 7 | 21.1 ± 0.6[a] | Neg | Neg | Neg | Neg | Neg |
| 22 ± 2 | 14 | 32.6 ± 1.3[b] | Neg | Neg | Neg | Neg | Neg |
| 22 ± 2 | 21 | 34.8 ± 2.0[c] | Neg | Neg | Neg | Neg | Neg |

[a] The theoretical limit of detection of the PED virus isolation method used was estimated to be able to detect as low as 1.0 viral particles/g.

[b] PEDV Ct values with uncommon superscripts within temperature by time storage condition differ ($P < 0.05$).

[c] Neg = negative or not detectable.

viruses like PRRSV and PRV (Polo et al., 2005) and a non-enveloped virus like SVD (Pujols et al., 2007). These studies demonstrated that the commercial spray drying conditions used for the manufacturing process of commercial SDP should be sufficient to eliminate low-medium heat resistant viruses like PEDV. The normal processing temperature condition used by commercial SDP manufacturing plants is >80 °C throughout its substance. The present data indicated that drying at 70 °C throughout its substance was already adequate to inactivate at least 5.15 log10 TCID50/g of this virus. Therefore, the heat treatment used during the commercial spray-drying process is theoretically more than adequate to inactivate PEDV if present in the raw material. Recent data would support such conclusion (Gerber et al., 2014; Opriessnig et al., 2014). In the first study, researchers used raw plasma from a non-infected pig spiked with wild type US strain of PEDV and either fed directly as raw liquid spiked plasma or spray-dried it (in a laboratory drier at 83 °C outlet temperature) and fed to naïve pigs. Their results indicated that the raw liquid spiked plasma was infective but the spray dried spiked plasma was not infective, indicating that spray drying at outlet temperature around 80 °C should be enough to inactivate the PEDV (Gerber et al., 2014). In the second study, piglets received a diet with 5% SDPP containing 5.1 ± 0.1 log10 PEDV RNA copies/g and showed no evidence of infectivity by the existing PEDV RNA present in the SDPP lot utilized (Opriessnig et al., 2014).

PEDV viremia has been reported only once in gnotobiotic pigs experimentally infected with PEDV genogroup 2 (Jung et al., 2014). It is usually accepted that PEDV RNA detected in serum is possible at the peak of disease, but viremia is reported to be short and of low magnitude (Pensaert, 1999). Therefore, it appears unlikely that PEDV viremia and utilization of blood from viremic pigs is a main source of PEDV contamination of SDPP. Probably, the presence of this virus in raw blood is more likely attributed to carcass contamination at time of slaughter (Gerber et al., 2014; Opriessnig et al., 2014).

The present data studying the survival of PEDV inoculated on SDBP appeared to be dependent upon storage temperature and time, with virus survivability associated with lower temperature storage conditions. However, virus concentration diminished considerably for the samples stored at 4 °C or 12 °C for 7 days compared to samples immediately stored frozen at −80 °C. Importantly, samples stored at room temperature did not contain infective PEDV by 7 days of storage, which is consistent with previous data reported by other researchers. They demonstrated that PEDV-positive feces spread evenly on the bottom surface aluminum tray and treated at 20 °C for 7 days were non infective when feed-back to naïve pigs by oral-gastric tube (Thomas et al., 2014). Also, it is important to highlight that, even at 4 °C, the virus was not infectious in cell culture after 21 days of storage. The fact that spray dried blood products are powders with low moisture (below 8%) and water activity (<0.6) are important, since PEDV does not survive very long under dry conditions (OIE, 2014).

The Ct values for samples stored at 4, 12 and 22 °C for 7 days were 21.59 ± 0.73, 22.18 ± 1.08 and 21.12 ± 0.62, respectively. These results indicated that qRT-PCR Ct values were not different between samples ($p > 0.05$), independently of the storage conditions. However, differences between days ($p < 0.05$) at each temperature condition should be indicative of progressive genome degradation. Moreover, the qRT-PCR positive results compared to those generated by cell culture of the virus indicated that the former technique is not a good indicator to establish PEDV infectivity. However, it is also true that virus isolation might not be highly sensitive; therefore, a swine bioassay would be the most definitive way to demonstrate the putative infectivity of those qRT-PCR positive samples which were negative after inoculation of VERO cells.

## 5. Conclusions

In summary, results of the present work demonstrated that PEDV (at least the European prototype strain, CV777) was apparently inactivated by spray drying conditions mimicking those used by the spray-dried blood products industry. Moreover, if SDP would become contaminated

with PEDV infectious virus, the present data indicates that PEDV would not survive for 7 days when stored at room temperature (22 °C) or 21 days if stored at refrigerated temperature (4 °C).

## Conflict of interest

## Acknowledgments

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