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**Citation for published version:**

Polo, J, Opriessnig, T, O'Neill, KC, Rodríguez, C, Russell, LE, Campbell, JM, Crenshaw, J, Segalés, J & Pujols, J 2013, 'Neutralizing antibodies against porcine circovirus type 2 in liquid pooled plasma contribute to the biosafety of commercially manufactured spray-dried porcine plasma' *Journal of Animal Science*, vol. 91, no. 5, pp. 2192-8. DOI: 10.2527/jas.2012-5705

**Digital Object Identifier (DOI):**

[10.2527/jas.2012-5705](https://doi.org/10.2527/jas.2012-5705)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

*Journal of Animal Science*

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# JOURNAL OF ANIMAL SCIENCE

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*J ANIM SCI* 2013, 91:2192-2198.

doi: 10.2527/jas.2012-5705 originally published online March 11, 2013

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://www.journalofanimalscience.org/content/91/5/2192>



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# Neutralizing antibodies against porcine circovirus type 2 in liquid pooled plasma contribute to the biosafety of commercially manufactured spray-dried porcine plasma<sup>1</sup>

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**ABSTRACT:** Neutralizing antibodies (NA) inherently present in pooled plasma collected at commercial abattoirs may provide some protection against potential porcine circovirus type 2 (PCV2) infectivity of plasma. Moreover, these NA may also contribute to the biosafety of spray-dried porcine plasma (SDPP). The objective of the study was to characterize and quantify the PCV2 antibody neutralizing capacity in pooled liquid porcine plasma and SDPP samples collected from industrial spray-drying facilities located in the Southeast and Midwest regions of the United States and the Northeast region of Spain. In the United States, PCV2 NA was determined in 1 sample of pooled liquid plasma from commercial spray-drying plants in the Southeast and 1 from the Midwest region. Obtained results were compared with those of a plasma sample from a PCV2 vaccinated sow and 1 from a PCV2 antibody negative sow. In Spain, 15 pooled liquid porcine plasma samples and 10 SDPP samples were collected at a commercial spray-drying plant total and NA against PCV2 were determined. Results with pooled liquid porcine plasma from commercial spray-drying facilities in the United States indicated that NA titers against PCV2 in these

samples ( $\log_2$  8.33  $\pm$  0.41 and 9.0  $\pm$  0.0) were similar or greater than the plasma from the PCV2-vaccinated sow ( $\log_2$  6.33  $\pm$  0.41). The analysis of U.S. samples indicated that liquid plasma diluted to 1:256 ( $10^{-2.41}$ ) was able to neutralize between 100 to 200 PCV2 virus particles or about 4  $\log_{10}$  median tissue culture infective dose (TCID<sub>50</sub>) per milliliter. Similarly, samples from the Spanish pooled liquid plasma and the SDPP samples indicated an increased amount of NA activity against PCV2. Specifically, a dilution of  $10^{-2.47 \pm 0.33}$  of plasma was able to inactivate 100 PCV2 virus particles; therefore, the inactivation capacity of commercial liquid plasma was greater than  $10^4$  TCID<sub>50</sub>/mL. The calculated 90% reduction in infected cells because of NA in pooled plasma samples ( $\log_2$  8.2  $\pm$  0.38) was less ( $P < 0.05$ ) than in its concentrate form of SDPP (mean,  $\log_2$  10.2  $\pm$  0.85). In conclusion, PCV2 NA contained in liquid pooled plasma from market pigs was detected at greater concentrations than that from a vaccinated sow and that after spray-drying biological neutralizing activity was conserved, which implies that the inherent NA in liquid plasma may have an important role in the biosafety of commercially produced SDPP.

**Key words:** biosafety, neutralizing antibodies, porcine circovirus type 2, spray dried porcine plasma, virus neutralization

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J. Anim. Sci. 2013.91:2192–2198  
doi:10.2527/jas2012-5705

<sup>1</sup>We thank K. Lin (Iowa State University) and N. Navarro and E. Cano (CRESA) for their assistance with the laboratory work. This study has been partially financed by the programs CDTI (IDI-20080591 and IDI-20101014) of the Spanish Government.

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Received August 3, 2012.  
Accepted February 21, 2013.

## INTRODUCTION

Spray-dried porcine plasma (SDPP) is well recognized as a key ingredient in diets for weaned pigs (Nelssen et al., 1999) because it promotes improvements in growth and feed intake and reduces

postweaning diarrhea (Coffey and Cromwell, 2001; Torrallardona, 2010). Porcine circovirus type 2 (PCV2) is a small circular, nonenveloped, single-stranded DNA virus (Tischer et al., 1982) found in most swine worldwide (Allan and Ellis, 2000; Zhou et al., 2006) and is very resistant to physical and chemical inactivation procedures (Royer et al., 2001; Martin et al., 2008) and dry heat treatment (O'Dea et al., 2008). Therefore, there are concerns that PCV2 may not be completely inactivated in commercial SDPP. Patterson et al. (2010) demonstrated PCV2 seroconversion in pigs after oral gavage of laboratory produced SDPP that was derived from a pig experimentally infected with PCV2. Analytical results of commercial SDPP reported the presence of PCV2 DNA copies (Pujols et al., 2011b; Shen et al., 2011). However, recent studies demonstrated that commercial SDPP containing PCV2 DNA copies did not transmit PCV2 when fed to healthy pigs for long periods (Pujols et al., 2008; Shen et al., 2011).

The failure to demonstrate PCV2 transmission by feeding commercial SDPP has been partially attributed to the more robust thermodynamics of commercial spray driers that result in rapid dehydration and involves a minimum heat treatment of 80°C throughout its substance (Polo et al., 2005; Pujols et al., 2008; Shen et al., 2011). In addition, the high prevalence of neutralizing antibodies (NA) in pooled liquid plasma may also contribute to the lack of transmission (Pujols et al., 2011a). Pooling of NA is a recognized safety step in production of human plasma products (Williams and Khan, 2010). Therefore, our objectives were to evaluate the activity of NA against PCV2 in pooled liquid plasma and to estimate their potential contribution to the biosafety of commercial SDPP.

## MATERIALS AND METHODS

### Study 1

**Sample Collection Procedures and Storage.** The commercial pooled liquid plasma were obtained from federally inspected slaughterhouses that follows USDA guidelines related with animal care at the time of slaughter. Blood collection and processing at the slaughterhouse does not involve any modification of the slaughter, not in the method nor in time. Two commercial pooled liquid raw plasma samples (8% solids) from 2 federally inspected abattoirs located in the Southeast and the Midwest areas of the United States were collected in 50-mL conical tubes from tankers at the spray-drying facility. During slaughter, plasma is typically collected from multiple pigs, pooled, and held in tanks at each abattoir before transport in insulated tankers to spray-drying facilities. Each tanker contains plasma from approximately 9,000 to 10,000

pigs. Plasma samples were immediately frozen after collection and stored at -20°C. Samples were shipped frozen overnight on wet ice to the Veterinary Medicine Laboratory at Iowa State University (Ames, IA) where they were immediately stored at -80°C until analysis.

One PCV2 antibody positive plasma (PP) sample obtained from a sow vaccinated against PCV2 (1 mL intramuscularly; Ingelvac CircoFLEX; Boehringer Ingelheim Vetmedica, Inc., Ingelheim, Germany) 140 d before collection and 1 negative control (NC) plasma sample from a nonvaccinated sow that was negative for PCV2 antibodies as determined by ELISA (Nawagitgul et al., 2002) and PCV2 DNA as determined by real-time PCR (Opriessnig et al., 2003) were also used in this study. All samples were diluted at 1:10 with modified eagle medium (MEM) and 1% antibiotic and antimycotic (AB/AM) mixture and heat inactivated at 56°C for 30 min before making the serial 2-fold dilutions. The diluted plasma samples were then centrifuged at 3,200 × g for 10 min at 4°C and supernatants obtained were used for the different analytical determinations. All samples were analyzed in triplicate.

**Porcine Circovirus Type 2 Real-Time Quantitative PCR and ELISA.** Presence, amount, and genotype of PCV2 DNA in the plasma samples were determined by real-time PCR assays (Shen et al., 2011). The presence of anti-PCV2 antibodies was determined using an open reading frame 2-based PCV2 IgG ELISA (Nawagitgul et al., 2002). Pooled liquid plasma samples were diluted in normal saline solution to constant solids of 8% before testing. The sample-to-positive (S:P) ratio was calculated by dividing the sample optical density at 450 nm by the positive control optical density, and samples with an S:P ratio of 0.2 or greater were considered positive.

**Neutralizing Antibody Testing Procedure.** Two-fold serial dilutions, consisting of 100 µL plasma in MEM with 1% AB/AM on a 96-well cell culture plate with a starting dilution of 4 and then adding 100 µL of PCV2 (PCV2a ISUVDL 98-15237) inoculum at a rate of 100 fluorescent focus units to all wells, were made. After incubation in 5% CO<sub>2</sub> at 37°C for 30 min and 1, 3, or 6 h, 100 µL of PK15 cell suspension in MEM with 10% fetal bovine serum (FBS) and 1% AB/AM mixture were added to each well, and the plates were incubated in 5% CO<sub>2</sub> at 37°C for an additional 48 h. The cell monolayer was then treated with 300 mM D-glucosamine (Sigma Chemical Co., St. Louis, MO) prepared in a MEM suspension of 25 µL/well for 20 min. After treatment, the cell monolayer was washed once with MEM and replenished with 200 µL of fresh MEM supplemented with 10% FBS and 1% AB/AM mixture and incubated for another 16 h. After the additional 16 h of incubation, the cell monolayer was fixed in cold 80% acetone (50 µL/well). Fifty microliters of PCV2-monoclonal antibody

(Rural Technologies, Inc., Brooking, SD) diluted at 1:1,000 with PBS was added to all wells and incubated for 30 min at 37°C. The plates were washed with PBS, and then fluorescein isothiocyanate anti-mouse conjugate (KPL, Inc., Gaithersburg, MD) diluted at 1:100 in PBS was added to all wells (50 µL/well). The plates were then incubated for an additional 30 min at 37°C. All plates were washed 3 times with PBS and dried. Plates were read using a fluorescent microscope at a magnification of 100 or 40x. Cells with nuclear or cytoplasmic fluorescent staining or both were recorded as infected. The percentage of virus neutralization at each dilution was calculated according to this formula:  $[1 - (\text{number of positive cells of each serum dilution} / \text{mean number of positive cells in the negative control cells})] \times 100$ . The NA titer was expressed as the reciprocal of the last dilution in which a given sample reduced the number of PCV2-infected cells by 90%.

## Study 2

### *Sample Collection Procedures and Storage.*

Fifteen pooled liquid plasma samples were collected in 20 mL conical tubes during 5 consecutive d from the holding tank of liquid plasma at the Spanish commercial spray-drying facility. The plasma samples were collected each day at 3 different time intervals during the production cycle for adequate characterization of the daily production. Samples were taken from the plasma tank at the beginning, midpoint, and end of the daily production cycle. Like in the United States, the samples were collected from a tanker containing plasma from approximately 9,000 to 10,000 pigs. All plasma samples were immediately frozen after collection and stored at -25°C. Samples were transported frozen to the laboratory (Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona, Barcelona, Spain) where they were stored at -80°C until analyzed.

In addition, samples from 10 manufacturing batches of SDPP representing from 35,000 to 40,000 pigs per batch were analyzed for presence of total antibodies and NA against PCV2a and PCV2b. The dried samples were collected and tested independently from the liquid samples. These dried samples were previously solubilized in sterilized deionized water at a rate of 1:9 before mixing with the virus particles.

***Porcine Circovirus Type 2 Real-Time Quantitative PCR and Serology.*** Detection and amount of PCV2 DNA genome present in the pooled liquid raw plasma and SDPP samples were determined by a real-time PCR assay (Olvera et al., 2004). The presence of total antibodies to PCV2 in the commercial pooled liquid porcine plasma samples and diluted dried samples were

investigated by an immunoperoxidase monolayer assay (IPMA; Fort et al., 2007).

***Neutralizing Antibody Testing Procedure.*** Fifty microliters of serum tested (either from the liquid plasma or from the diluted dried plasma) were serially diluted 2-fold in 96-well plates from 1:20 to 1:20,480 in complete MEM (with 5% FBS, 100 mM L-glutamine, 100 U/mL of penicillin, 100 mg/mL streptomycin, and 3% nonessential amino acids). Fifty microliters at 200 median tissue culture infective dose ( $\text{TCID}_{50}$ ) of a PCV2a stock (Burgos strain 390/L8, known to react with antibodies elicited against PCV2b isolates; Kurtz et al., 2010) were added to each well. After 2 h of mixture incubation, 20,000 freshly trypsinized PK-15 cells were added to each well and incubated for 72 h at 37°C in 5%  $\text{CO}_2$ . Cells were fixed in absolute ethanol at -20°C for 30 min. Infected cells were then revealed by IPMA technique (Fort et al., 2007). Plates were read using a microscope at 100x amplification. Wells containing cells with nuclear, cytoplasmic, or both staining patterns were recorded as infected and affected cells were individually counted. The percentage of virus seroneutralization (%SNT) at each dilution was calculated according to this formula:  $\%SNT = [1 - (\text{mean number of positive cells of 2 replicas of each serum dilution} / \text{mean number of positive cells in negative control wells})] \times 100$ . To validate the plate, previous defined criteria were used (Fort et al., 2007), including positive and negative controls and back titration on virus inoculum. The NA titer was calculated as the reciprocal of the last dilution, in which a given serum sample was able to reduce the number of PCV2-infected cells by 90%. These values were designated as SNT90%.

### *Statistical Analyses*

Results of NA titers were expressed as mean  $\pm$  SD of the  $\log_2$  of the reciprocal plasma dilution. Analysis of variance among different samples (U.S. samples) or sampling periods during the day or between days of the liquid plasma sample collection (Spanish samples) was performed using the ANOVA simple analysis (Statgraphics Centurion XV; StatPoint Technologies, Inc., Warrenton, VA). Statistical differences of means were determined using LSD as an all pairwise multiple comparison procedure. The *t*-student test was used to compare the values between liquid and dried plasma of the Spanish samples. Differences were considered significant when  $P < 0.05$ .

## RESULTS

### *Study 1*

The anti-IgG PCV2 S:P ratios of the 2 pooled plasma samples collected from commercial spray-drying

facilities and the PP sample are summarized in Table 1. The NC samples did not contain measurable anti-PCV2 IgG antibodies. Antibodies to PCV2b was identified in both pooled commercial plasma samples ( $\log_{10}$  5.37 – 5.54 genomic copies per mL). The NC and PP samples did not contain PCV2 DNA.

Both commercial liquid plasma samples had NA titers greater than the positive control plasma sample from the PCV2 vaccinated sow ( $P < 0.05$ ), indicating that pooled plasma samples from commercial abattoirs contained greater concentrations of NA against PCV2 than concentrations expected in vaccinated animals (Table 1). The NA titers for the NC sample were less than  $2 \log_2$  (below the detection limit). Differences in respect to the incubation time between the plasma and the SNT90% titer were not statistically significant although numerically the results obtained after 1 h of incubation were greatest.

### Study 2

The total antibody titers against PCV2 in the liquid samples had an average of  $\log_2$   $12.79 \pm 1.00$  as measured by IPMA. The mean PCV2 genomic copies were  $\log_{10}$   $4.84 \pm 0.23/\text{mL}$ . The NA titers in these samples had an average SNT90% of PCV2 infected cells of  $\log_2$   $8.20 \pm 1.08$  (Table 2). The correlation ( $r = 0.143$ ) between total antibodies and NA in the liquid plasma was low.

No statistical differences between total or NA titers against PCV2 were observed during the 5 consecutive days tested. Also, no differences for total or NA titers were observed by sampling intervals (beginning, midpoint, and end) of the manufacturing process.

In the SDPP samples from Spain (Table 3), the average total antibody titers were  $14.52 \pm 0.26 \log_2$  and  $10.30 \pm$

**Table 1.** Porcine circovirus type 2 (PCV2) ELISA sample-to-positive (S:P) ratios and virus neutralizing antibody (NA) titers at different times of in vitro exposure in commercial pooled liquid plasma samples from the Southeast and Midwest slaughter regions of the United States

Sample <sup>1</sup>	PCV2 ELISA S:P ratio	PCV2 NA by time <sup>2</sup>			
		30 min	1 h	3 h	6 h
NC	0.000	<2	<2	<2	<2
PP	0.784	$5.3 \pm 0.4$	$6.3 \pm 0.4$	$5.7 \pm 0.4$	$5.7 \pm 0.4$
Southeast	0.895	$8.0 \pm 0.0$	$8.3 \pm 0.4$	$7.7 \pm 0.4$	$7.7 \pm 0.4$
Midwest	0.758	$8.0 \pm 0.0$	$9.0 \pm 0.0$	$8.0 \pm 0.0$	$8.3 \pm 0.4$

<sup>1</sup>NC is the negative control plasma sample from a sow that was not vaccinated with PCV2 vaccine; PP is the positive control plasma sample from a sow that was vaccinated with PCV2 vaccine; Southeast is pooled liquid plasma from market pigs slaughtered at abattoirs located in the Southeast region of the United States; Midwest is pooled liquid plasma from market pigs slaughtered at abattoirs located in the Midwest region of the United States.

<sup>2</sup>The NA titers are expressed as the mean of  $\log_2$  of the reciprocal of the serum dilution  $\pm$  SEM able to reduce the virus replication by 90% over various incubation time intervals. Neutralizing antibody titers were determined by fluorescence focus neutralization assay.

$1.06 \log_2$  for SNT90% NA. The correlation ( $r = 0.014$ ) between total antibodies and NA was low but similar to liquid plasma. In SDPP samples, the mean PCV2 genomic copies/mL was  $\log_{10}$   $3.53 \pm 0.20$ . Titers in SDPP were greater ( $P < 0.001$ ) for both IPMA and NA compared with the values obtained from the liquid plasma.

## DISCUSSION

Pooling plasma is a recognized safety step in the production of certain human plasma products (Solheim et al., 2000, 2006, 2008) because of successful neutralization of antigens in the presence of corresponding antibodies, especially for stable viruses that are difficult to inactivate by other methods (Solheim et al., 2008; Williams and Khan, 2010).

Although a single pig may not have antibodies to all pathogens present in the population, a batch of commercially produced SDPP is represented by the pooled plasma from approximately 6,000 to 10,000 pigs. Therefore, it is likely that this pooled plasma contains antibodies and NA against all pathogens circulating in the pig population at any point in time. Several authors (Pujols et al., 2011a; Shen et al., 2011) suggested that, in addition to the thermal dehydration by spray-drying liquid plasma, the inherent NA against specific viruses of concern in liquid plasma should contribute to the biosafety of commercial SDPP.

Dietary spray-dried plasma improves postweaning feed intake and growth of pigs (Coffey and Cromwell,

**Table 2.** Immunoperoxidase monolayer assay (IPMA) antibody titers to porcine circovirus type 2 (PCV2) and virus neutralizing antibody (NA) titers against PCV2 at 90% of viral neutralization endpoint (SNT90%) in commercial pooled liquid porcine plasma samples collected by day of production and at different time intervals within day of production at a drying facility in Spain

Sample <sup>1</sup>	IPMA titers for PCV2 antibody <sup>2</sup>	PCV2 NA SNT90% <sup>3</sup>
d 1	$12.0 \pm 0.4$	$8.7 \pm 1.5$
d 2	$12.3 \pm 0.0$	$7.7 \pm 0.8$
d 3	$13.0 \pm 0.8$	$8.3 \pm 0.4$
d 4	$13.7 \pm 0.8$	$8.0 \pm 0.7$
d 5	$13.0 \pm 0.8$	$8.3 \pm 0.4$
Beginning	$13.1 \pm 0.6$	$7.6 \pm 0.3$
Middle	$12.1 \pm 0.2$	$8.2 \pm 0.4$
End	$13.1 \pm 0.6$	$8.8 \pm 0.7$

<sup>1</sup>Pooled liquid plasma samples were collected at the Spanish drying facility for 5 consecutive d at the beginning, middle (after half of the production run), and end of the production run (just before the plasma deposit was empty).

<sup>2</sup>Immunoperoxidase monolayer assay antibody titers to PCV2 (expressed as mean of  $\log_2$  of the reciprocal of the serum dilution  $\pm$  SEM) for each production day and within production day sampling period.

<sup>3</sup>Neutralizing antibody at 90% of viral neutralization end point (expressed as mean of  $\log_2$  of the reciprocal of the serum dilution  $\pm$  SEM) for each production day and within production day sampling period.

2001; Van Dijk et al., 2001; Torrallardona, 2010) as well as productive measures for lactating sows and their progeny (Crenshaw et al., 2007). Based on current evidence, the use of SDPP in animal feed is considered safe and there are numerous steps in the manufacturing process of commercial SDPP that contribute to the biosafety of this functional protein ingredient. Only blood from healthy pigs that have passed antemortem inspection by veterinary competent authorities and approved as fit for slaughter for human consumption is collected for commercially produced SDPP. Avoidance of collecting plasma from clinically affected pigs decreases the risk of potential pathogen transmission. Other steps such as spray drying have demonstrated their efficacy as a pasteurization-like process to eliminate different bacteria and viruses (Pujols et al., 2008). Studies have shown the effectiveness of spray drying to inactivate bacterial coliforms (Polo et al., 2002) as well as viruses of importance to the swine industry such as porcine reproductive and respiratory syndrome virus, pseudorabies virus (Polo et al., 2005), or swine vesicular disease virus (Pujols et al., 2007). Design and configuration of the spray-drying process affects microbial survival; therefore, it may not be appropriate to extrapolate and interpret data obtained from a laboratory spray drier to that of a commercial spray dryer (Thybo et al., 2008). As a consequence, it is not recommended to conduct safety studies of heat-resistant viruses, such as porcine parvovirus (PPV) or PCV2, based on material produced by a laboratory spray drier. In fact, these machines have less capability of viral inactivation compared with commercial spray driers because of

various factors, such as droplet size, shear forces, drying temperatures, and dwell time of particles inside the drier (Shen et al., 2011). For these heat resistant viruses, it is recommended to conduct safety studies by feeding naïve pigs with diets containing commercial SDPP at greater inclusion levels and for a 3 to 4 times longer duration than typical commercial conditions. Several studies have indicated that SDPP does not transmit either PPV or PCV2 (Polo et al., 2005; Pujols et al., 2008, 2011a; Shen et al., 2011), which are 2 of the most heat and chemically resistant swine viruses.

Neutralizing antibodies seem to be essential to clear PCV2 infection in pigs, and low titers of PCV2 NA have been linked to the occurrence of postweaning multisystemic wasting syndrome (PMWS), which is associated with PCV2 infectivity (Meerts et al., 2006; Fort et al., 2007). Also, PCV2 vaccination induces PCV2 NA in piglets and may prevent detectable PCV2 viremia after challenge (Fort et al., 2008). Thus, available data indicate that PCV2 virus particles are neutralized by PCV2 NA and development of PMWS is reduced or prevented. Because both vaccinated and naturally infected PCV2 pigs usually have greater NA titers than NC, it is expected that their presence in commercially collected pooled liquid blood or plasma would neutralize PCV2 virus particles that might be present in the blood from subclinically infected pigs.

The present results with pooled liquid porcine plasma collected from commercial abattoirs in the United States indicated that PCV2 NA titers in these samples were similar or greater than titers detected from a single PCV2 vaccinated sow 140 d after vaccination. This result indicates that both PCV2 infection and extensive PCV2 vaccination protocols used in the United States contribute to the high presence of PCV2 NA detected in slaughter pigs. Similarly, positive titers of PCV2 NA were detected in samples of pooled liquid porcine plasma collected from a commercial spray-drying plant in Spain, which is consistent with the results in grower–finisher pigs selected from 34 different farms located in northeastern Spain (Fort et al., 2007). Also, the presence of positive titers of total antibodies against PCV2 were detected in 278 samples of SDPP from daily batches collected over a 14 mo period (November 2009 to December 2010) at Spanish manufacturing plants (Polo et al., 2011). The Spanish SDPP samples came from 25 abattoirs, which represented >60% of the total Spanish pig production.

The results of the Spanish samples indicated a consistent presence of total and NA against PCV2 with minimal day-to-day or within-day variation in both liquid and spray-dried porcine plasma. In addition, results indicated that total PCV2 antibody titers in SDPP also had low variability as demonstrated by the small SD of the 10 manufactured lots tested. This is in agreement with

**Table 3.** Immunoperoxidase monolayer assay (IPMA) antibody titers against PCV2 at 90% of viral neutralization endpoint (SNT90%) in commercial spray-dried porcine plasma samples from Spain

Lot number <sup>1</sup>	IPMA PCV2 antibody titers <sup>2</sup>	PCV2 NA SNT90% <sup>3</sup>
Y024542	14.3 ± 0.0	10.0 ± 0.6
Y024652	14.8 ± 0.7	9.0 ± 0.0
Y024912	14.3 ± 0.0	9.7 ± 0.3
Y025022	14.3 ± 0.0	10.3 ± 0.3
Y025132	14.3 ± 0.0	10.0 ± 0.6
Y025242	14.8 ± 0.7	9.0 ± 0.0
Y025352	14.8 ± 0.7	11.7 ± 0.3
Y025462	14.8 ± 0.7	10.7 ± 0.3
Y025612	14.3 ± 0.0	11.0 ± 0.0
Y025722	14.3 ± 0.0	10.7 ± 0.3

<sup>1</sup>Ten consecutive manufacturing lots of spray-dried porcine plasma samples were collected at a drying facility in Spain.

<sup>2</sup>Immunoperoxidase monolayer assay antibody titers to PCV2 (expressed as mean of log<sub>2</sub> of the reciprocal of the serum dilution ± SEM) for the different manufacturing lots.

<sup>3</sup>Neutralizing antibody at 90% of viral neutralization end point (expressed as mean of log<sub>2</sub> of the reciprocal of the serum dilution ± SEM able to reduce the virus replication by 90%) for the different manufacturing lots.

Borg et al. (2002), who reported that samples of spray-dried plasma collected during a whole year of production from different manufacturing plants located in the United States, Argentina, and Spain contained a consistent and significant amount of antibody titers against a variety of specific pathogenic bacteria (*Escherichia coli* K88, *E. coli* K99, *Salmonella typhimurium*, and *Clostridium botulinum*). Recently, Shen et al. (2011) reported that all 116 liquid, concentrated, and spray-dried U.S. commercial plasma samples contained positive titers of PCV2 antibodies. These data are consistent with the ubiquitous nature of PCV2 (Allan and Ellis, 2000; Walker et al., 2000; Zhou et al., 2006).

The present study with pooled plasma samples from the United States indicates that liquid plasma diluted 1:256 ( $10^{-2.41}$ ) was able to inactivate between 100 to 200 TCID<sub>50</sub> of infectious PCV2 virus particles, indicating that PCV2 NA in pooled plasma from commercial abattoirs could inactivate around  $10^4$  TCID<sub>50</sub>/mL of PCV2 virus particles. In the instance of the liquid plasma from Spain, it was found that a dilution of  $10^{-2.47} \pm 0.33$  of plasma was able to inactivate 100 TCID<sub>50</sub> of PCV2 virus particles. Therefore, the inactivation capacity of liquid pooled plasma collected from commercial slaughter pigs is greater than  $10^4$  TCID<sub>50</sub>/mL of virus. The inherent NA in liquid plasma can be recognized as an effective safety step according to the established guidelines for virus validation studies for human plasma (EMEA, 1996, 2001; Millipore, 2002). These guidelines define a unit operation biosafety step as effective, ineffective, or moderately effective. Effective steps provide a reduction factor of at least 4 logs<sub>10</sub> and are unaffected by small perturbations in process variables. Ineffective steps provide a reduction factor of 1 or less, and moderately effective steps fall between these 2 extremes. Therefore, the results of the present studies indicate that inherent NA in liquid pooled plasma can be considered an effective biosafety step in the manufacturing process of SDPP.

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