Lack of transmission of porcine circovirus type 2 to weanling pigs by feeding them spray-dried porcine plasma

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An experiment was conducted to determine whether spray-dried porcine plasma containing 2·47 x 10^5 DNA copies of porcine circovirus type 2 (PCV-2) could infect weanling pigs when fed to them. Five specific pathogen-free (SPF) weanling pigs were fed ad libitum for 45 days a control diet and six pigs were fed a test diet containing 8 kg SDPP per 100 kg feed. The two groups were housed in separate biosecurity level-3 rooms. None of the pigs in either group developed any clinical signs or became PCV-2 viraemic or seroconverted.

SPRAY-DRIED porcine plasma (SDPP) is used widely in diets for weanling pigs to improve their growth and feed efficiency (Coffey and Cromwell 2001). Blood from healthy animals is normally sterile, except in the case of animals with subclinical bacteraemia or viraemia (Bourgeois and Le Roux 1982, Ockerman and Hansen 1994, Carretero and Parés 2000). There are several safety steps in the manufacture of SDPP, starting with the fact that only animals deemed healthy and suitable for human consumption by government inspectors are the source of the blood. In addition, the blood is pooled from many animals slaughtered on the same day, resulting in a diverse mixture of antibodies (Borg and others 2002) that would be expected to have at least a partial neutralising effect on potential pathogens in the plasma. The blood is collected into containers with anticoagulant, chilled and centrifuged to separate the plasma from the cellular fraction. The plasma is then spray dried to produce a powdered ingredient for food, feed and industrial applications. Spray-drying can be considered similar to pasteurisation because there are rapid changes in temperature and pressure during the process followed by the rapid dehydration of the product (Polo and others 2005, Pujols and others 2007). Spray drying reduces the number of viable microorganisms (Lievense 1991, Polo and others 2002), provided that the process is carried out under established conditions to ensure reductions in the microbial load (Lian and others 2002, Polo and others 2002). The physical treatment is therefore detrimental to the viability of viruses and bacteria.

Porcine circovirus type 2 (PCV-2) is a small, single-stranded DNA virus belonging to the Circoviridae family, which is ubiquitous in domestic pigs and wild boar worldwide (Segalés and others 2005). It is considered to be the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS), which causes worldwide economic losses for the pig industry (Segalés and others 2005). There are few data about its biological and physicochemical characteristics, but porcine circovirus type 1 has a buoyant density of 1·37 g/ml in caesium chloride, is resistant to inactivation at pH 3 and by chloroform, and is stable at 70°C for 15 minutes (Allan and others 1994). It is probable that these properties also apply to PCV-2. Moreover, PCV-2 is very resistant to disinfection (Royer and others 2001, Martin and others 2007).

Owing to the highly resistant nature of PCV-2 and the fact that SDPP is a commercially manufactured by-product, it is possible that it could transmit PCV-2 when fed to pigs. The objective of this study was to evaluate whether commercially manufactured SDPP, positive for the PCV-2 genome by quantitative PCR, could transmit PCV-2 when fed to specific pathogen-free (SPF) weanling pigs.

MATERIALS AND METHODS

Eleven SPF Landrace pigs with a mean (sd) weight of 6·6 (1·1) kg, three to four weeks of age from four different sows were obtained from a high-health-status genetic selection farm. Pigs from this farm were free from porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV), *Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Brachyspira hyodisenteriae* and the specific *Pasteurella multocida* and *Bordetella bronchiseptica* responsible for atrophic rhinitis. The sows on the farm had low antibody titres to PCV-2 and were monitored routinely for the absence of PCV-2 viraemia. The piglets used in the study came from sows that were not viraemic to PCV-2 and had low antibody titres, thus diminishing the risk of maternal transfer of infection. At the beginning of the study, the pigs were not PCV-2 viraemic and they had no antibodies to PRRSV, ADV or swine vesicular disease virus (SVDV), but they did have detectable antibodies to PCV-2. The absence of PCV-2 antibodies was not required because maternal antibodies are widespread in commercial weanling pigs.

The pigs were identified with ear tags and allocated by weight, sex and maternal origin, to a group of five control pigs and a group of six test pigs. Each group was housed in an independent isolated room. All the pigs were fed the control diet ad libitum for 11 days before the experimental period began.

Dietary treatments

The experimental diets were formulated to contain similar amounts of crude protein, lysine and metabolisable energy, and were provided in mash form. No growth-promoting antimicrobials or medications were included in the pigs' feed or water during the study. The control diet was devoid of any animal protein. The test diet contained 8 per cent SDPP and was fed ad libitum for 45 days. This percentage was chosen to provide more than twice the usual amount of SDPP consumed by pigs fed commercial diets; the common practice is to supplement feed with 2 to 6 per cent SDPP for 14 to 21 days after weaning.

The commercially manufactured SDPP (AP820P batch Y617932; APC Europe) used in the experimental test diet contained 2·47 x 10^5 PCV-2 DNA copies/ml when measured by real-time quantitative PCR (Olvera and others 2004) and had an antibody titre to PCV-2 of 1/5120, as measured by immunoperoxidase monolayer assay (IPMA) (Rodríguez-Arrioja and others 2000). This batch was chosen because it contained the largest number of PCV-2 DNA copies among five commercial batches.

Animal facilities and monitoring

The two groups of pigs were housed in separate isolation rooms with biosecurity level 3 facilities. The rooms were

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TABLE 1: Seroneutralisation (SN) 50 per cent antibody titres to porcine circovirus type 2 (PCV-2) on day 0 of the experiment and immunoperoxidase monolayer assay (IPMA) antibody titres to PCV-2 on all the sampling days (both expressed as dilutions) in the five control pigs and six test pigs

Group of pigs	SN titre Day 0	IPMA titre Day 0	Day 10	Day 35	Day 45
Control	1/64	1/320	<1/20	<1/20	<1/20
Control	1/16	1/20	1/20	<1/20	<1/20
Control	1/8	1/80	<1/20	<1/20	<1/20
Control	1/16	1/80	1/80	1/20	1/20
Control	1/32	1/80	1/20	1/20	1/20
Test	1/8	1/80	1/80	<1/20	<1/20
Test	1/32	1/320	1/20	<1/20	1/20
Test	1/32	1/80	<1/20	<1/20	<1/20
Test	1/16	1/320	1/80	1/20	1/20
Test	1/16	1/80	1/20	1/20	<1/20
Test	1/32	1/320	1/20	<1/20	<1/20

equipped with a HEPA-filtered air installation and security decontamination system for organic residues, and the pigs were placed in a thoroughly cleaned and disinfected isolation box. Specific clothing and clinical material were used in each room and the animal attendants had a soapy shower between rooms. The temperature in the isolation boxes was controlled by thermostat and mechanical ventilation, initially to 24 to 26°C, and after two weeks to 20 to 22°C. Artificial lighting followed a circadian rhythm of 24 hours: 11 hours 45 minutes of continuous light and 11 hours 45 minutes of continuous darkness, with a 15 minute gradient of light between each period. Laboratory animal laws and experimental procedures approved by the CReSA animal experimentation ethics committee were followed. Water and feed were supplied ad libitum by means of an automatic system of water troughs and feed hoppers.

Any signs of pain or behavioural changes, reductions in feed intake or respiratory signs (scoring from 0 to 3 for all parameters: 0, none; 1, slight; 2, moderate; and 3, severe) were recorded daily for each individual pig throughout the 45 days. If a score of more than 1 was observed in any pig, the rectal temperatures of all the pigs in the group were measured. If a pig had a rectal temperature over 40·5°C, appropriate treatment and diagnostic procedures were considered.

The pigs were weighed on days 0, 10 and 45 and bled on days 0, 10, 35 and 45. On day 45 they were euthanased and examined postmortem.

Laboratory procedures

Before it was analysed, the SDPP used in the test diet was reconstituted in sterile distilled water at a concentration of 9 per cent w/v to obtain a similar concentration to that of liquid porcine plasma.

The samples of serum collected and the SDPP used in the test diet were analysed for the genome of PCV-2 by a standard PCR technique (Quintana and others 2002).

The presence of antibodies to PCV-2 in the experimental pigs was investigated by three different tests: 1) a virus seroneutralisation (SN) technique (Fort and others 2007) on serum samples collected on day 0; 2) IPMA (Rodríguez-Arrioja and others 2000) on all the serum samples collected; and 3) a capture ELISA for the detection of specific IgG and IgM antibodies to PCV-2 (INGEZIM CIRCOVIRUS IgG/IgM; INGENASA) on all the serum samples collected. The SN was performed only on day 0 to determine the capacity of the maternal immunity to neutralise a potential infection by PCV-2 at the beginning of the study. The IPMA and ELISA tests, as more sensitive techniques, were used to assess the putative seroconversion of the pigs to PCV-2 during the experiment.

Tests for antibodies to PPV, PRRSV and ADV were also applied to samples of serum collected on days 0 and 45 by indirect ELISAs: INGEZIM PPV (INGENASA), HerdChek PRRS 2XR Antibody Test Kit (IDEXX Laboratories) and INGEZIM ADV TOTAL (INGENASA). Finally, the presence of SVDV neutralising antibodies in sera collected on days 0 and 45 was investigated by using published protocols (OIE 2004).

Statistical analyses

Descriptive statistics were derived for the bodyweight, average daily weight gain, clinical scores and serological results for the two groups. For statistical testing, the non-parametric Mann-Whitney U test was applied to the bodyweight and average daily weight gain because of the small numbers of animals in the study. NCSS software (Hintze 2004) was used for the statistical analyses.

RESULTS

The mean (sd) bodyweights of the control and test pigs on day 0 were 8·7 (1·9) and 7·7 (1·2) kg, and on day 45 they were 42·5 (4·4) and 38·7 (3·7) kg, respectively. There was no significant difference between the groups. The pigs did not show any adverse clinical signs and no therapeutic interventions were required.

The treated and control pigs did not become PCV-2 viraemic during the study, and the ELISA results showed that all of them remained seronegative throughout. However, at the beginning of the study all the pigs in both experimental groups had some level of antibody titres detected by IPMA and SN techniques, ranging from 1/20 to 1/320, and from 1/8 to 1/64, respectively (Table 1). These antibodies are considered to have been of maternal origin because the titres decreased in both groups over time, reaching low or negative titres by day 10 (range <1/20 to 1/80); IPMA antibody titres to PCV-2 have been described as negative or low for values up to 1/80, medium from 1/320 to 1/1280, and high for titres of more than 1/5120 (Rodríguez-Arrioja and others 2000, Vincente and others 2004). The PCR and serological results therefore showed that the pigs did not become PCV-2 viraemic and did not seroconvert. However, the PCV-2 ELISA was not as sensitive as the IPMA technique.

DISCUSSION

It has been reported that there are improvements in the growth rate, feed intake and feed efficiency of weanling pigs fed diets containing SDPP (Coffey and Cromwell 2001, Van Dijk and others 2001, Pierce and others 2005). This was not the case in this study; however, a sample size greater than that used in this study is required to find statistical differences in body growth parameters.

Maternal antibodies have been reported to play a role in preventing the development of PMWS, but they are not able to prevent the establishment of subclinical PCV-2 infection or seroconversion (Meerts and others 2006, Fort and others 2007). These studies showed that pigs with neutralising maternal antibody titres similar to those observed on day 0 of this experiment were unable to avoid becoming infected or seroconverting when infected experimentally with PCV-2. The presence of residual maternal antibodies to PCV-2 at the beginning of the experiment represents fairly well the usual field conditions under which SDPP is used. In fact, the PCV-2 antibody titres in the pigs at three weeks of age were lower than the titres observed in pigs under commercial conditions (Rodríguez-Arrioja and others 2002). The fact that in this experiment the SDPP contained large numbers of DNA copies of PCV-2 and the pigs had low levels of maternally derived antibodies reinforces the low probability of SDPP as a source of infectious PCV-2.

On days 0 and 45 of the experiment, all the pigs were also seronegative to PRRSV, ADV and SVDV. On day 0, one control and two test pigs were seropositive to PPV (range 1/400 to 1/800); however, these antibodies were considered to have been of maternal origin because their titre steadily decreased throughout the study (from 1/200 to 1/400 on day 45). Maternal antibodies to PPV can persist in pigs up to three to six months of age (Mengeling 2006).

The results agree with previous studies in which no seroconversion to several viruses (PPV, PRRSV, PRV and PCV-2) was observed when pigs were fed diets containing SDPP (Polo and others 2005, Nofrarías and others 2006). The SDPP used by Nofrarías and others (2006) was analysed and found to contain only 10^3 to 10^4 PCV-2 DNA copies/ml, far fewer than the SDPP used in this study.

Carasova and others (2007) reported that the quantity of PCV-2 in pig serum is age dependent. They found a mean PCV-2 genome load of 10^5 copies/ml by 19 to 25 weeks of age, the usual slaughter age range in Spain. Therefore, the PCV-2 load in the batch of SDPP used was as would have been expected. However, the PCV-2 genome was quantified by real-time PCR, which does not distinguish between infectious and non-infectious virus particles (Wang and others 2004). Consequently, the potential infectiveness of SDPP cannot be totally discounted on the basis of the results of this study. However, the primary objective of the study was to determine whether SDPP was a potential source of PCV-2 infection to piglets when administered in feed, which is how SDPP is used under normal production conditions.

The lack of PCV-2 infectivity in the pigs could be attributed to the partial inactivation of PCV-2 in commercially manufactured SDPP, as a result of antigen-antibody interactions in the liquid plasma before spray drying and/or during the spray drying process. This idea is supported by the fact that spray drying can inactivate several log titres of other pig viruses, including PRRSV, ADV and SVDV (Polo and others 2005, Pujols and others 2007).

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