RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of the safety and efficacy of a second generation live chimeric PCV1-2 vaccine in the PCV2-PRRSV coinfection model – **NPB #08-132**

Investigator: Tanja Opriessnig, Dr med vet, PhD

Institution: Iowa State University

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Industry Summary:

The efficacy of a live chimeric porcine circovirus (PCV) type 1-2 vaccine based on subtype PCV2a was evaluated in a PCV2b and porcine reproductive and respiratory syndrome virus (PRRSV) coinfection model. Eighty-three, 2-week-old pigs were randomized into 12 treatment groups including eight vaccinated and four control groups. Pigs were vaccinated intramuscularly or orally at 3 weeks of age followed by inoculation with PCV2b and PRRSV at 7 weeks of age. PCV1-2a vaccination elicited an anti-PCV2-IgG response which was delayed in pigs vaccinated orally. Intramuscular vaccination significantly reduced PCV2b viremia compared to non-vaccinated pigs. The results indicate that PCV1-2a vaccination induced protective immunity against PCV2 in pigs experimentally coinfected with PCV2b and PRRSV and the intramuscular route of vaccination is more effective than oral.

Contact Information: tanjaopr@iastate.edu

Keywords: Porcine circovirus type 2 (PCV2); Porcine reproductive and respiratory syndrome virus (PRRSV); Coinfection; Live vaccination; Efficacy

Scientific Abstract:

The efficacy of a live chimeric porcine circovirus (PCV) type 1-2 vaccine based on subtype PCV2a was evaluated in a PCV2b and porcine reproductive and respiratory syndrome virus (PRRSV) coinfection model. Eighty-three, 2-week-old pigs were randomized into 12 treatment groups including eight vaccinated and four control groups. Pigs were vaccinated intramuscularly or orally at 3 weeks of age followed by inoculation with PCV2b and PRRSV at 7 weeks of age. PCV1-2a vaccination elicited an anti-PCV2-IgG response which was delayed in pigs vaccinated orally. Intramuscular vaccination significantly reduced PCV2b viremia compared to non-vaccinated pigs. The results indicate that PCV1-2a vaccination induced protective immunity against PCV2 in pigs experimentally coinfected with PCV2b and PRRSV and the intramuscular route of vaccination is more effective than oral.

Introduction

Porcine circovirus (PCV) can be divided into two main genotypes: PCV type 1 (PCV1) and PCV type 2 (PCV2) (Allan et al., 1999b). PCV1 was initially identified as a cell culture contaminant of the porcine kidney cell line PK-15 (Tischer et al., 1974) and is non-pathogenic in swine (Tischer et al., 1986; Allan et al., 1995). In contrast, PCV2 is pathogenic and associated with a number of diseases in swine including reproductive failure (O'Connor et al., 2001; Madson et al., 2009) and post-weaning clinical manifestations such as wasting, respiratory disease, diarrhea, pallor, porcine dermatitis and nephropathy syndrome (PDNS), and jaundice (Allan et al., 2000b; Harms et al., 2002).

PCV2 is a small, non-enveloped, single-strand DNA virus with a circular genome of 1,767 to 1,768 nt in size (Mankertz et al., 1997). It belongs to the genus Circovirus and family *Circoviridae* (Allan et al., 2000a; Todd et al., 2005). The viral genome of PCV2 consists of two major open reading frames (ORFs) (Fenaux et al., 2000; Mankertz et al., 2000): ORF1 encodes viral replication proteins (Rep and Rep'), and ORF2 encodes the immunogenic capsid protein (Nawagitgul et al., 2000). A third ORF, ORF3 has been described and is thought to be involved in apoptosis of lymphocytic and hepatic cells (Liu et al., 2006).

Coinfection of pigs with PCV2 and porcine parvovirus (PPV) (Allan et al., 1999a; Kennedy et al., 2000), PCV2 and *Mycoplasma hyopneumoniae* (Opriessnig et al., 2004) and PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV) (Allan et al., 2000b; Rovira et al., 2002) have been shown to increase PCV2 replication and the severity of clinical disease. Among all co-infecting pathogens, PRRSV is the most commonly identified virus in field cases of PCVAD (Pogranichniy et al., 2002) and evidence suggests this combination of pathogens substantially increases severity of disease in pig production systems (Harms et al., 2001; Harms et al., 2002; Kim et al., 2003; Morandi et al., 2010).

Prior to commercial PCV2 vaccines becoming widely available, PCVAD caused significant economic losses (Clark, 1997; Allan et al., 2000a). However, with the approval and extensive use of several inactivated or subunit PCV2 vaccines, those losses have been minimized on many farms and pig production systems across the world (Fachinger et al., 2008; Kixmöller et al., 2008; Horlen et al., 2008; Cline et al., 2008; Segalés et al., 2009). Among currently available commercial vaccines there is one inactivated vaccine based on a chimeric PCV1-2 virus, two subunit vaccines based on the PCV2 capsid protein expressed in the baculovirus system, and one inactivated vaccine based on a full-length PCV2. All the currently available products are based on PCV2a which has been shown to be cross-reactive against PCV2b in several studies (Fort et al., 2008; Opriessnig et al., 2008b).

The chimeric PCV1-2a virus was developed by replacing the ORF2 of PCV1 with that of PCV2 within the genomic backbone of the non-pathogenic PCV1 (Fenaux et al., 2002). There was no evidence of reversion of the PCV1-2 chimera virus to its parental wild-type PCV1 or PCV2 virus after eleven serial passages in PK-15 cells, and the vaccine was found to be genetically stable after being recovered from pigs during three serial passages (Gillespie et al., 2008). In addition, the vaccine was shown to be attenuated in pigs, inducing protective immunity in the PCV2a challenge model (Fenaux et al., 2004). Recently, the vaccine efficacy of selected commercial vaccines and the experimental live chimeric PCV1-2a vaccine were compared in a triple challenge model (PCV2b, PPV, and PRRSV) using commercial pigs that had variable levels of anti-PCV2

antibodies and variable levels of viremia (Shen et al., 2010). All vaccination regimes were found to be able to reduce the amount of PCV2 DNA in serum, reduce PCV2-associated microscopic lesions, and improve average daily weight gain compared to non-vaccinated challenged pigs (Shen et al., 2010).

Potential advantages of using a live vaccination include inducing better immune responses due to activation of cellular immunity in addition to humoral immunity and some live vaccines can be administered via the drinking water thereby reducing risk (needle breakage) and cost (labor, needles) associated with intramuscular administration of vaccines. The objectives of this study were to determine the efficacy of a live chimeric PCV1-2a vaccine in the PCV2b-PRRSV coinfection model and to compare intramuscular and oral routes of vaccination.

Objectives

a. To determine the efficacy of a live chimeric PCV2 vaccine in the PCV2-PRRSV coinfection model.
b. To determine the safety of the vaccine product; PRRSV has been shown to enhance PCVAD in our well defined experimental model.

Materials and Methods

2.1 Animals and housing

Eighty-three, 21-day-old, colostrum-fed, cross-bred conventional pigs were obtained from a herd confirmed to be free of PCV2, PRRSV, and swine influenza virus (SIV) by routine serological testing. The pigs were weaned at 2 weeks of age and transported to the Livestock Infectious Disease Isolation Facility at Iowa State University, Ames, Iowa. On the day of arrival, pigs were randomly assigned to one of 12 groups and eight rooms. Non-vaccinated (four rooms) and vaccinated groups (four rooms) were separated according to treatment status (PRRSV, PCV2, PRRSV and PCV2, non-infected pigs). Within each room, pigs were contained in one (non-vaccinated rooms) to two (vaccinated rooms; pigs were separated into pens by vaccine administration route; the pens were located at different sides of the room; access to the pens required changing of the outerwear between pens) raised wire decks equipped with one nipple drinker and one self-feeder. All groups were fed ad libitum with a balanced, pelleted, feed ration free of animal proteins (excluding whey) and antibiotics (Nature's Made, Heartland Coop).

2.2. Experimental design.

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (8-08-6618-S). The experimental groups are summarized in Table 1.

Table 1
Experimental design.

Group designation	n	Vaccination Route	Inoculation	
			PCV2	PRRSV
None-Negative	7	-	-	-
None-PCV2	7	-	Yes	-
None-PRRSV	7	-	-	Yes
None-PCV2-PRRSV	7	-	Yes	Yes
IM-Negative	7	Intramuscularly	-	-
IM-PCV2	7	Intramuscularly	Yes	-
IM-PRRSV	7	Intramuscularly	-	Yes
IM-PCV2-PRRSV	7	Intramuscularly	Yes	Yes
PO-Negative	6	Orally	-	-
PO-PCV2	7	Orally	Yes	-
PO-PRRSV	7	Orally	-	Yes
PO-PCV2-PRRSV	7	Orally	Yes	Yes

All pigs were confirmed to be PCV2 seronegative by ELISA prior to the start of the animal experiments. Twenty-eight days before inoculation (-28 days post inoculation or dpi), pigs in the vaccinated groups received a PCV1-2a live vaccine orally (n=27) or intramuscularly (n=28). A portion of the vaccinated and non-vaccinated pigs were inoculated with PCV2b (n=21), PRRSV (n=21) or PCV2b and PRRSV (n=21) on 0 dpi and necropsy was conducted at 21 dpi. Between -28 dpi and 21 dpi, blood was collected from all pigs on a weekly basis in 8.5 ml serum separator tubes (Fisher Scientific Inc.). The blood was centrifuged at 2000 *x g* for 10 min at 4°C and serum was stored at -80°C until testing. Serum samples were tested for levels of anti-PCV2 IgG antibody, anti-PRRSV-IgG antibody, PCV1-2a DNA, PCV2 DNA, and PRRSV RNA. Tissues collected during necropsy were analyzed by immunohistochemistry (IHC) to determine the amount of PCV2 antigen.

2.3. Clinical evaluation

All pigs were weighed on the day of arrival and at vaccination, challenge and necropsy. The average daily weight gain was calculated before (-28 to 0 dpi), after (0 to 21 dpi) PCV2b-PRRSV inoculation, and for the entire study period (-28 to 21 dpi).

2.4. Vaccination

The pigs were vaccinated at -28 dpi using an experimental live chimeric PCV1-2a (Fenaux et al., 2000; Fenaux et al., 2002) vaccine at a dose of $0.5 \times 10^{3.5}$ median tissue culture infective dose ($TCID_{50}$) per ml. Intramuscular (IM) vaccination was done by injecting 2 ml PCV1-2a vaccine into the right side of the neck using a 0.7 mm × 25.4 mm needle (Kendal Monoject™, Gosport PO13, OAS, Tyco Healthcare U.K. LTD., United Kingdome) and a 3 ml syringe (Fisher Scientific, Inc.). For the oral (PO) vaccination, the pig was held in the upright position and the vaccine was administered by slowly dripping 2 ml into the mouth of each pig using a 3 ml syringe (Fisher Scientific, Inc.).

2.5. PCV2b inoculation

The PCV2b isolate NC-16845 was propagated on PK-15 cells and was used at an infectious dose of 0.25×10^4 $TCID_{50}$ per ml. Each pig (n=42) in the PCV2 inoculated groups (Table 1) received 1 ml of the inoculum IM into the right neck area and 3 ml (1.5 ml per nostril) intranasally (IN) by holding the pig in the upright position and administering the inoculum by slowly dripping 1.5 ml in each nostril using a 3 ml syringe (Fisher Scientific, Inc.).

2.6. PRRSV Inoculation

PRRSV isolate ATCC VR2385 (Halbur et al., 1995) was propagated on MARC-145 cells to a final titer of $1\times10^{5.0}$ $TCID_{50}$/ml. Each pig (n=42) in the PRRSV inoculated groups (Table 1) received 2.5 ml of the PRRSV inoculum intranasally in a similar fashion as described for PCV2 inoculation.

2.7. Serology

All serum samples from all groups were tested for anti-PCV2 Ig-G antibodies using the SERELISA® PCV2 Ab Mono Blocking kit (Synbiotics Europe, Lyon, France) according to the manufacturers' instructions. The results were expressed as sample to negative corrected (SNc) ratio, and samples were considered to be negative if the SNc ratio was greater than 0.50 and positive if the SNc ratio was less than or equal to 0.50.

2.8. RNA and DNA extraction

Viral DNA and RNA were extracted from serum samples using the MagMax™ Viral RNA and DNA Multi-Sample isolation Kit (Applied Biosystems, Life Technologies, Carlsbad, California, USA) on the Kingfisher Flex System (ThermoFisher Scientific, Pittsburgh, Pennsylvania, USA) according to the instructions of the manufacturer.

2.9. Detection of PCV2 and PCV1-2 RNA by quantitative real-time PCR

Serum samples collected at 0, 7, 14, and 21dpi were tested for the presence of PCV2 and PCV1-2 DNA by quantitative real-time PCR assay using primer-probe combinations as described (Opriessnig et al., 2003; Shen et al., 2010). The following modifications were used for both assays: a different commercially available master mix (TaqMan® Fast Universal PCR Master Mix, Applied Biosystems) was used, the reaction volume was 25µl, only one aliquot was tested for each sample and the thermal cycler conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min.

2.10. Detection of PRRSV RNA by quantitative reverse transcriptase real-time PCR

Serum samples collected at 7, 14 and 21 dpi were tested for the presence and amount of PRRSV RNA as described (Shen et al., 2010).

2.11. Necropsy

All pigs were humanely euthanized by intravenous pentobarbital sodium overdose (Fatal Plus®, Vortech Pharmaceuticals, LTD, Dearborn, Michigan, USA) and necropsied at 21 dpi. The total extent of macroscopic lung lesions (ranging from 0 to 100%) was estimated and scored (Halbur et al., 1995). The size of superficial inguinal lymph nodes were compared among groups (Opriessnig et al., 2006). Sections of lymph nodes (superficial inguinal, external iliac, mediastinal, tracheobronchial, and mesenteric), tonsil, heart, thymus, kidney, colon, spleen, liver, small (ileum) and large intestine (spiral colon) were collected at necropsy, fixed in 10% neutral-buffered formalin, and routinely processed for histological examination.

2.12. Histopathology

Microscopic lesions were evaluated by two veterinary pathologists (TO, PGH) blinded to the treatment groups. Lung sections were scored for the presence and severity of interstitial pneumonia, ranging from 0 (normal) to 6 (severe diffuse) (Halbur et al., 1995). Sections of heart, liver, kidney, ileum, colon and thymus were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 (none) to 3 (severe). Lymph nodes, spleen, and tonsil were evaluated based on lymphoid depletion (LD) and histiocytic replacement (HR) of follicles, ranging from 0 (normal) to 3 (severe) (Opriessnig et al., 2004). The overall lymphoid lesion score was calculated as described (Opriessnig et al., 2004). In brief, a combined scoring system for each lymphoid tissue that ranged from 0 to 9 (lymphoid depletion score 0-3; granulomatous inflammation score 0-3; PCV2 immunohistochemistry or IHC score 0-3) was used. The scores (lesions and PCV2-IHC) of the seven lymphoid tissues ([lymph node pool] × 5, spleen, and tonsil) were added together and divided by 7. The lymph node pool consisted of one section each of tracheobronchial, superficial inguinal, external iliac, mediastinal, and mesenteric lymph nodes.

2.13. Immunohistochemistry

Immunohistochemistry (IHC) for detection of PCV2-specific antigen was performed on selected formalin-fixed and paraffin-embedded sections of lymph nodes (superficial inguinal, external iliac, mediastinal, tracheobronchial, and mesenteric), tonsil, and spleen using a rabbit polyclonal antiserum (Sorden et al., 1999). PCV2 antigen scoring was done by a veterinary pathologist (TO) blinded to animal group designation. Scores ranged from 0 (no signal) to 3 (more than 50% of lymphoid follicles contained cells with PCV2 antigen staining) (Opriessnig et al., 2004).

2.14. Statistical analysis

For data analysis, JMP® software version 8.0.1 (SAS Institute, Cary, NC) was used. Summary statistics were calculated for all the groups to assess the overall quality of the data set including normality. Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) for continuous data (log_{10} transformed PCR data, ELISA data, weight gain, lung scores). A p-value of less than 0.05 was set as the statistically significant level. If found to be significant, pairwise test using Tukey's adjustment was performed to determine which groups differed. Real-time PCR results (copies per ml of serum) were log_{10} transformed

prior to statistical analysis. Non-repeated nominal data (histopathology scores, IHC scores, and lymph nodes size) were assessed using a non-parametric Kruskal-Wallis one-way ANOVA, and if there was a significant difference, pairwise Wilcoxon tests were used to evaluate differences among groups. Differences in prevalence were determined by using chi-square tests. Percent reduction for PCV2 amount was determined as follows: 100 − [(100 × mean $\log_{10}$ genomic copies/ml in the vaccinated group) ÷ (mean $\log_{10}$ genomic copies/ml in positive control animals)].

Results

3.1. Average of daily weight gain

There were no significant differences ($p>0.05$) among all groups in body weight at -28, 0 or 21 dpi. Vaccination did not impact the average daily weight gain from -28 to 0 dpi and there were no differences between non-vaccinated pigs (n=28; 31.8±1.9 lb), pigs vaccinated with PCV1-2a orally (n=27; 32.8±1.6 lb) and pigs vaccinated with PCV1-2a IM (n=28; 33.2±1.5 lb). In addition, there was no difference in average daily weight gain between -28 and 0 dpi and -28 and 21 dpi (data not shown). Specifically, evaluation of the average daily weight gain from 0 to 21 dpi revealed the following: among groups inoculated with PCV2 there was no difference regardless of vaccination status (None-PCV2, 33.3±2.2 lb; IM-PCV2, 32.2±2.0 lb, PO-PCV2, 33.5±3.0 lb). Among groups inoculated with PCV2 and PRRSV there was no difference regardless of vaccination status (None-PCV2-PRRSV, 26.0±1.6 lb; IM-PCV2-PRRSV, 33.4±3.6 lb, PO-PCV2-PRRSV, 29.7±2.3 lb). Among groups that remained non-inoculated there was no difference regardless of vaccination status (None, 32.9±1.9 lb; IM-None, 33.5±2.2 lb, PO-None, 35.4±1.8 lb). Similarly, among groups inoculated with PRRSV there was no difference regardless of PCV2 vaccination status (None-PRRSV, 29.4±2.3 lb; IM-PRRSV, 31.6±3.3 lb, PO-PRRSV, 33.3±2.7 lb).

3.2. Anti-PCV2 IgG antibody levels

All non-vaccinated animals (None-Negative, None-PCV2, None-PRRSV, None-PCV2-PRRSV) remained seronegative for PCV2 until 7 dpi and groups not infected with PCV2 (None-Negative, None-PRRSV) remained seronegative for PCV2 until 21 dpi. After IM PCV1-2a vaccination, PCV2 seroconversion was first observed at 2 weeks post vaccination at which time (-14 dpi) 7.1% (2/28) of the pigs seroconverted. By -7 dpi, 53.6% (15/28) of the pigs were PCV2 seropositive and by 0 dpi, 75.0% (21/28) of the pigs were seropositive. After oral PCV1-2 vaccination, seroconversion was first seen at 4 weeks post vaccination (0 dpi) in 3.7% (1/27) of the pigs; non-PCV2 inoculated groups (PO-Negative, PO-PRRSV) had 23.1% (3/13), 76.9% (10/13), and 69.2% (9/13) seropositive pigs at 7, 14, and 21 dpi, respectively (Table 2).

3.3. PCV1-2 viremia

PCV1-2 DNA was not detected in any of the pigs at 0, 7, 14, and 21 dpi (data not shown).

3.4. Prevalence and amount of PCV2 DNA in serum

PCV2 DNA was not detected in any serum samples collected at 0 dpi or in any of non-PCV2 infected groups (None-Negative, None-PRRSV, IM-Negative, IM-PRRSV, PO-Negative, PO-PRRSV) at 7, 14 and 21 dpi (data not shown). In non-vaccinated pigs (None-PCV2, None-PCV2-PRRSV) 85.7% (12/14), 100% (14/14) and 100% (14/14) of the pigs were viremic at 7, 14, and 21 dpi, respectively. In pigs IM PCV1-2a vaccinated, 21.4% (3/14) pigs were viremic on each 7, 14, and 21 dpi. In pigs orally PCV1-2a vaccinated, 71.4% (10/14), 78.6% (11/14), and 71.4% (10/14) of the pigs were viremic at 7, 14, and 21 dpi, respectively. Compared to the non-vaccinated groups, viremia was reduced in the IM PCV1-2a groups by 79.2% (7 dpi), 84.6% (14 dpi) and 80.4% (21 dpi). For PO-PCV1-2a groups, viremia compared to the non-vaccinated pigs was reduced by 24.6% (7 dpi), 20.8% (14 dpi) and 29.6% (21 dpi).

3.5. Prevalence and amount of PRRSV RNA in serum

PRRSV RNA was detected only in the groups inoculated with PRRSV and 100% (42/42) of the PRRSV inoculated animals were positive for PRRSV RNA on 7, 14, and 21 dpi. The group $\log_{10}$ PRRSV RNA means were not different among groups (data not shown).

3.6. Gross lesions

Macroscopic lesions were characterized by lungs that failed to collapse, were mottled tan and occasionally had cranioventral tan consolidation (particularly in pigs infected with PRRSV) with the following group mean scores: None-Negative, 0.0±0.0; None-PCV2, 0.0±0.0; None-PRRSV, 10.0±2.4; None-PCV2-PRRSV, 20.3±6.8; IM-Negative, 0.0±0.0; IM-PCV2, 3.1±2.5; IM-PRRSV, 10.3±3.8; IM-PCV2-PRRSV, 0.3±0.3; PO-Negative, 0.0±0.0; PO-PCV2, 0.9±0.9; PO-PRRSV, 0.3±0.3; and PO-PCV2-PRRSV, 5.0±3.6. None-PCV2-PRRSV infected pigs had significantly ($p<0.05$) higher lung lesion scores compared to all groups except IM-PRRSV and None-PRRSV. Lymph node sizes ranged from being normal to 2 times the normal size without differences among groups.

3.7. Microscopic lesions

Microscopic lung lesions were characterized by mild-to-moderate focal-to-multifocal interstitial pneumonia characterized by type 2 pneumocyte hypertrophy and hyperplasia and increased numbers of lymphocytes and macrophages in alveolar septa walls. In general, the lesions appeared to be in the resolving stages. Lymphoid lesions were characterized by mild-to-severe histiocytic replacement of primary or secondary follicular nodes and by mild-to-severe lymphoid depletion in lymph nodes, tonsil, and spleen tissues. In general, PCV2-asscoiated lesions were mild (overall lymphoid score range 0 to 3) in IM-PCV2, IM-PCV2-PRRSV groups, mild-to-moderate (overall lymphoid score range 0 to 6) in PO-PCV2, PO-PCV2-PRRSV, and None-PCV2 groups and mild-to-severe (overall lymphoid score range 0-8) in the None-PCV2-PRRSV group. The group mean overall lymphoid score was 0.10±0.10 for None-Negative, 2.14±0.69 for None-PCV2, 0.37±0.24 for None-PRRSV, 3.00±0.87 for None-PCV2-PRRSV, 0.06±0.06 for IM-Negative, 0.81±0.37 for IM-PCV2, 0.57±0.50 for IM-PRRSV, 0.87±0.37 for IM-PCV2-PRRSV, 0.00±0.00 for PO-Negative, 3.14±0.78 for PO-PCV2, 0.51±0.25 for PO-PRRSV and 0.93±0.51 for PO-PCV2-PRRSV.

3.8. PCV2 antigen in tissues as determined by IHC

PCV2 antigen was not detected in any of the non-PCV2 infected pigs. The prevalence of PCV2 IHC positive animals was as follows: None-PCV2, 42.9% (3/7); None-PRRSV-PCV2, 71.4% (5/7); IM-PCV2, 14.3% (1/7); IM-PCV2-PRRSV, 57.1% (4/7); PO-PCV2, 71.4% (5/7); and PO-PCV2-PRRSV, 14.3% (1/7).

Discussion

Advances in molecular techniques such as clone development and DNA recombination led to the production of a chimeric PCV1-2 infectious clone (Fenaux et al., 2002) that is currently being used in an inactivated form and licensed for growing pigs 3 weeks and older (Suvaxyn® PCV2, Pfizer Animal Health, Inc) and can be used as a one-dose or two-dose application. All of the commercially available PCV2 vaccines to date are inactivated products and require one to two doses of intramuscular administration which is costly and labor intensive. One other disadvantage of using an inactivated product is that inactivated vaccines are often less immunogenic compared to live virus resulting in the need to utilize adjuvants or give multiple doses in order to achieve long-term vaccine efficacy. The objective of this study was to test the efficacy of a live version of the commercial available PCV1-2 product in the PCV2b-PRRSV coinfection model using two different administration routes, intramuscularly and orally.

In previous studies using the live PCV1-2a infectious clone, it was found that pigs developed a protective immunity against PCV2a (Fenaux et al., 2004). The majority of the piglets seroconverted to PCV2 between 28 and 35 dpi, and although not all the animals had seroconverted by the time of challenge, they were all protected against subsequent PCV2a challenge, suggesting that high PCV2 antibody responses are not entirely necessary for protection (Krakowka et al., 2002; Darwich et al., 2003; Fenaux et al., 2003; Fenaux et

al., 2004). In our study, the majority of intramuscularly vaccinated pigs (75%; 21/28) had seroconverted four weeks after vaccination which is in agreement with Fenaux et al. (2004). However, in the groups that were vaccinated orally, only 3.6% (1/28) pigs seroconverted four weeks after vaccination. Nevertheless, as evident from the non-infected PO-Negative control group, antibodies in this group continued to increase afterwards indicating a delayed antibody response against PCV2.

PCV1-2 viremia was not identified in any of the vaccinated pigs as measured by a real-time PCR specific for PCV1-2 targeting the area of recombination of PCV1 and PCV2 (Shen et al., 2010). This finding agrees with the results found by Fenaux et al. (2004). In addition, coinfecting pathogens such as PRRSV are known to increase PCV2 replication (Harms et al., 2001; Rovira et al., 2002; Opriessnig et al., 2008a) and the absence of PCV1-2 viremia in PRRSV infected pigs (IM-PRRSV, IM-PCV2-PRRSV, PO-PRRSV, PO-PCV2-PRRSV) as well as the absence of PCV2 antigen in tissues of vaccinated but not PCV2-inoculated pigs (IM-Negative, IM-PRRSV, PO-Negative, PO-PRRSV) further emphasizes the decreased virulence of the chimeric PCV1-2a and the safety of this experimental product.

In this study, PCV2b was detectable after challenge in all PCV2 inoculated treatment groups. In contrast, previously PCV2 DNA was not detectable in vaccinated animals after challenge (Fenaux et al., 2003; Fenaux et al., 2004). This may be due to the fact that we used a different detection method for PCV2 which is more sensitive compared to what was previously used (real-time PCR versus gel-based PCR). In the current study, there were significant differences in prevalence and amount of PCV2 DNA with a reduction of PCV2 viremia ranging from 79.2% to 84.6% in the pigs vaccinated intramuscularly compared to non-vaccinated pigs. In addition, only 21.4% of pigs vaccinated by this route were PCV2 viremic after PCV2 challenge. Moreover, PCV2 viremia was also reduced in pigs vaccinated orally; however, the vaccine efficacy in the PO-PCV2 and PO-PCV2-PRRSV groups was not as impressive as that in the IM-PCV2 and IM-PCV2-PRRSV groups. PCV2 viremia was reduced ranging from 20.8% to 29.6% in pigs vaccinated orally compared to non-vaccinated groups with a prevalence of PCV2 viremia ranging from 71.4% to 78.6 % between 7 and 21 dpi. The observed difference in vaccine efficacy may have to do with the interval between vaccination and challenge (4 weeks); as the oral vaccination seemed to have a delayed antibody response suggesting that a longer interval between vaccination and challenge may be required for induction of protective immunity with this administration route.

One novel aspect of the current study is the usage of a coinfection challenge model. Dual-infection with PCV2b and PRRSV was used because PRRSV is considered to be one of the major pathogens involved in the porcine respiratory disease complex (Harms et al., 2001; Rovira et al., 2002; Opriessnig et al., 2008a) with PCV2. In this study, PRRSV viremia occurred in 100% of the animals in all the groups infected with PRRSV and was detectable by 7 dpi. Previous research with the live PCV1-2a chimeric virus has not been conducted with concurrent infections. Concurrent PRRSV infection did not reduce vaccine efficacy as evidenced by similar amounts of PCV2 DNA in both PCV2 and PCV2-PRRSV groups. In addition, PRRSV did not enhance PCV1-2 replication as PCV1-2 was not detectable at any of the time points tested (0, 7, 14 and 21 dpi).

Another novel aspect of this study is the evaluation of the oral route for usefulness of application of the live PCV1-2a vaccine. Previously, intra-hepatic, intra-lymphoid and intramuscular routes have been described (Fenaux et al., 2003; Fenaux et al., 2004; Gillespie et al., 2008). Even though the chimeric PCV1-2a candidate vaccine has been demonstrated to produce a protective immune response, further investigations are necessary to better understand the duration of live PCV1-2a-induced humoral and cellular immunity. In the field, PCV2 vaccination breaks are often perceived to occur in the late finisher stages. However, experimental studies often focus on pigs less than 10 weeks of age instead of using older animals and the studies conducted are most often short term. This and other studies have shown a protective response after intramuscular vaccination; though, the oral route should still be investigated more thoroughly especially in order to evaluate long term vaccine response under re-exposure or re-challenge conditions which are more likely to mimic what is going on under field situation.

In summary, we evaluated a live PCV1-2a second generation vaccine candidate in the PCV2b-PRRSV coinfection model and found that the vaccine used was highly efficacious when used intramuscularly. More data need to be generated to better understand the research findings in this area, and to further evaluate the oral route of vaccine administration. Oral vaccination did result in a protective immunity of lower magnitude and delayed

onset compared to the intramuscular administration. It is possible, that a higher dose of vaccine antigen may be necessary to improve performance of the orally administered product.

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