RESEARCH REPORT
SWINE HEALTH

Title: Comparison of the efficacy of vaccines based on subtype PCV2a or PCV2b in their ability to protect against PCV2b or PCV2a/b challenge – **NPB #11-055**

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Date Submitted: June 27, 2012

Industry Summary

Essentially all of the current commercial porcine circovirus type 2 (PCV2) vaccines are based on genotype 2a (PCV2a) and are effective in protecting against PCV2b challenge which is the current predominant genotype in the global pig population. The objective of this study was to compare PCV2a- and PCV2b-based on their ability to control PCV2b viremia under experimental conditions. Sixty-three pigs were randomly assigned to one of eight groups. At day 0 (D0), 16 pigs were vaccinated with an experimental live-attenuated chimeric PCV2 vaccine based on genotype 2a and 16 with genotype 2b. Challenge was done at D28 using PCV2b or a combination of PCV2a and PCV2b, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine parvovirus (PPV) to mimic what commonly occurs in the field. The experiment was terminated at D49. Pigs vaccinated with PCV1-2b had significantly higher levels of PCV1-2 viremia and shedding of PCV1-2 in feces and nasal secretions but also a more robust humoral immune response as evidenced by significantly higher ELISA S/P ratios compared to PCV1-2a vaccination. Regardless of challenge, PCV1-2b vaccination significantly reduced the prevalence and amount of PCV2 viremia compared to PCV1-2a vaccination. Interestingly, in non-vaccinated pigs concurrent PCV2a infection resulted in clinical disease in 1/8 pigs and increased macroscopic lung lesions compared to pigs challenged with PCV2b alone.

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Keywords: Porcine circovirus type 2; Vaccine efficacy; Genotypes; Piglets; PCV2a/PCV2b coinfection.

Scientific Abstract:

Essentially all of the current commercial porcine circovirus type 2 (PCV2) vaccines are based on genotype 2a (PCV2a) and are effective in protecting against PCV2b challenge which is the current predominant genotype in the global pig population. The objective of this study was to compare PCV2a- and PCV2b-based on their ability to control PCV2b viremia under experimental conditions. Sixty-three pigs were randomly assigned to one of eight groups. At day 0 (D0), 16 pigs were vaccinated with an experimental live-attenuated chimeric PCV2 vaccine based on genotype 2a and 16 with genotype 2b. Challenge was done at D28 using PCV2b or a combination of PCV2a and PCV2b, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine parvovirus (PPV) to mimic what commonly occurs in the field. The experiment was terminated at D49. Pigs vaccinated with PCV1-2b had significantly higher levels of PCV1-2 viremia and shedding of PCV1-2 in feces and nasal secretions but also a more robust humoral immune response as evidenced by significantly higher ELISA S/P ratios compared to PCV1-2a vaccination. Regardless of challenge, PCV1-2b vaccination significantly reduced the prevalence and amount of PCV2 viremia compared to PCV1-2a vaccination. Interestingly, in non-vaccinated pigs concurrent PCV2a infection resulted in clinical disease in 1/8 pigs and increased macroscopic lung lesions compared to pigs challenged with PCV2b alone further supporting the idea that concurrent PCV2a/PCV2b infections is necessary for optimal PCV2 replication.

Introduction

Porcine circovirus (PCV) type 2 (PCV2) is currently one of the most important viruses affecting the global pork industry. PCV2 was first recognized as contributing to disease in pigs of high health status shortly after weaning in the late 1990s (Harding et al., 1997). Since then, PCV2 has been identified in all major pork producing regions and when uncontrolled is associated with high economic losses. Porcine circovirus associated disease (PCVAD) may manifest as diarrhea, systemic disease along with wasting, pneumonia, or reproductive failure (Opriessnig et al., 2007a). During 2005 and 2006, a newly recognized PCV2 variant, genotype PCV2b, spread rapidly across North American swine production regions devastating producers with high mortality and morbidity (Cheung et al., 2007). Interestingly, a worldwide shift in PCV2 genotype from PCV2a towards PCV2b was identified around the time of the severe PCVAD outbreaks (Cheung et al., 2007; Gagnon et al., 2007; Patterson et al., 2010).

The worldwide prevalence of PCVAD associated with the complex pathogenicity of the disease led to the rapid development and implementation of control strategies. Several commercial PCV2 vaccines are now available for swine producers to minimize losses associated with PCVAD (Opriessnig et al., 2007a; Patterson et al., 2010). PCV2 vaccines have been shown to reduce clinical disease, PCV2 viremia and lesions associated with PCV2 in the field (Fachinger et al., 2008; Kixmöller et al., 2008; Horlen et al., 2008; Takahagi et al., 2009; Martelli et al., 2011; Fraile et al., 2012). Under experimental conditions, PCV2 vaccines have been shown to stimulate neutralizing antibodies, overcome passively-derived antibodies, improve growth performance parameters, and reduce fecal and nasal shedding of PCV2 (Opriessnig et al., 2008a; Opriessnig et al., 2009; Fort et al., 2009; Beach et al., 2010). In the USA, PCV2 vaccines were initially introduced in 2006 and have since become one of the most commonly administered vaccines used in growing pigs in North America. Among commercial PCV2 vaccines available in the USA, two vaccines are based on the capsid protein encoded by open reading frame (ORF) 2, which is expressed in the baculovirus system. The other currently available vaccine is based on a chimeric PCV1-2 by using ORF1 which encodes the replicase gene from the non-pathogenic PCV type 1 (PCV1) as backbone and ORF2 of PCV2 (Fenaux et al., 2003; Fenaux et al., 2004b). All commercial PCV2 vaccines used in North America today are based on PCV2a.

The commercially available chimeric PCV1-2a vaccine is the inactivated version of an experimental live-attenuated chimeric PCV2 vaccine which has been proven to be non-pathogenic and effective in the growing pig model (Fenaux et al., 2004a; Thomas et al., 2007; Shen et al., 2010b; Opriessnig et al., 2011a). Serially passage of the live chimeric PCV1-2a in cell culture and pigs showed its stability (Gillespie et al., 2008). Although it has been demonstrated that initial infection with PCV2a confer cross protection against subsequent PCV2b challenge (Fort et al., 2008; Opriessnig et al., 2008b), a live-attenuated chimeric PCV2

vaccine based on genotype 2b has also been developed recently (Beach et al., 2010). The live-attenuated chimeric PCV1-2b vaccine was effective against PCV2b challenge (Beach et al., 2010; Opriessnig et al., 2011b; Hemann et al., 2012) and also demonstrated cross protection again PCV2a (Beach et al., 2011). Today, PCV2b is the predominant genotype in the global pig population (Patterson et al., 2010). To the authors' knowledge, a direct side-by side comparison of the efficacies of both genotype 2a and 2b-based PCV2 vaccines has not been conducted to date.

Objective

The objective was to determine if there are differences between PCV2 vaccines based on subtype of the PCV2 (PCV2a versus PCV2b) in the vaccine as measured by their ability to control PCV2b viremia in the PCV2a-PCV2b-PRRSV-PPV challenge model in conventional pigs.

Materials and Methods

2.1. Animals, housing, and experimental design

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC #11-09-6831-S) and the Institutional Biosafety Committee (IBC #09-I-0030-A). Sixty-three, 14 to 35-day-old specific-pathogen-free pigs were obtained from a commercial swine herd free of PCV2, PRRSV and swine influenza virus as determined by routine serology. Breeding animals were vaccinated against PPV. At arrival, the pigs were blocked by age and randomly divided into eight treatment groups of 7 to 8 pigs and 8 rooms as outlined in Table 1.

Table 1. Experiment design. PCV1-2a or PCV1-2b vaccination was done on trial day 0 and challenge with a combination of PCV2a, PCV2b, PRRSV and PPV was done on trial day 28.

Group designation	Pig No	Vaccination*	Challenge**
VAC/2a-Ch/2b-PRRSV-PPV	8/7	PCV1-2a	PCV2b, PRRSV, PPV
VAC/2b-Ch/2b-PRRSV-PPV	8	PCV1-2b	PCV2b, PRRSV, PPV
Ch/2b-PRRSV-PPV	8	None	PCV2b, PRRSV, PPV
Negative controls	7	None	None
VAC/2a-Ch/2a-2b-PRRSV-PPV	8	PCV1-2a	PCV2a, PCV2b,PRRSV,PPV
VAC/2b-Ch/2a-2b-PRRSV-PPV	8	PCV1-2b	PCV2a,PCV2b, PRRSV, PPV
Ch/2a-2b-PRRSV-PPV	8	None	PCV2a, PCV2b, PRRSV, PPV
Ch/PRRSV-PPV	8/7	None	PRRSV, PPV

*Intramuscularly with 1 ml live-attenuated chimeric vaccine virus.
**Intranasally with 5 ml of PCV2b or 5 ml of PCV2b and 5 ml of PCV2a, 1 ml PPV, and 2.5 ml PRRSV.

At day 0 (D0), 32/63 pigs were vaccinated and at D28 all pigs except negative controls were challenged with PCV2b or a combination of PCV2a and PCV2b as outlined in Table 1. In addition, all pigs except negative controls were also challenged with PRRSV and PPV. At D49, all pigs were euthanized, a necropsy was conducted, macroscopic lesions were evaluated, and tissues were collected for histopathology. Blood was collected on a weekly basis in 8.5 ml serum separator tubes (BD vacutainer®, BD Biosciences), centrifuged at 3220 × *g* for 10 min at 4ºC and the serum was stored at -80ºC until testing. Nasal and fecal swabs were collected on a weekly basis using sterile swabs (Fisherbrand applicators, Fisher Scientific, Inc.) and stored in 1 ml of sterile saline solution (Fisher Scientific Inc.) at -80ºC until testing. Serum samples were tested for presence of antibodies against PCV2, PRRSV and PPV and serum samples, nasal and fecal swabs were tested for presence and amount of PCV1-2 DNA, PCV2 DNA, PPV DNA and PRRSV RNA.

2.2. Clinical evaluation

Following the challenge, the pigs were individually monitored daily for clinical signs, including sneezing, lethargy and coughing. At D0, D35 and D49 all pigs were weighed.

2.3. Vaccination

The construction and *in vivo* characterization of the two experimental live-attenuated PCV1-2a and PCV1-2b vaccines used in the current study has been described previously (Fenaux et al., 2004b; Beach et al., 2010). Both vaccines have been shown to be attenuated and efficacious in previous experimental studies (Beach et al., 2010; Opriessnig et al., 2011b). The PCV1-2a and PCV1-2b vaccines utilized had an infectious titer of $10^{3.5}$ and 10^{4} 50% tissue infective dose ($TCID_{50}$) per ml, respectively. Each pig in the vaccinated groups received 1 ml PCV1-2a (VAC/2a-Ch/2b-PRRSV-PPV; VAC/2a-Ch/2a-2b-PRRSV-PPV) or 1 ml PCV1-2b (VAC/2b-Ch/2b-PRRSV-PPV; VAC/2b-Ch/2a-2b-PRRSV-PPV) intramuscularly in the right neck area at D0.

2.4. Challenge

PCV2a isolate ISU-40865 (Fenaux et al., 2003) and PCV2b isolate NC-16845 (Opriessnig et al., 2011b) were propagated in PCV-free PK-15 cells until a $TCID_{50}$ of $10^{4.5}$ per ml was reached. PRRSV isolate ATCC VR2385 was propagated on MARC-145 cells. The 7th passage of the virus was used which had a titer of $10^{5.0}$ $TCID_{50}$ per m. (Huang et al., 2009). The PPV challenge virus stock was from passage 4 in pigs (10% tissue homogenate suspension containing lungs of aborted fetuses in minimal essential medium) from the year 1982 and was used at an approximate titer of $10^{4.9}$ $TCID_{50}$ per ml (Opriessnig et al., 2011b). Each pig received 5 ml PCV2b or 5 ml PCV2a and 5 ml PCV2b, 2.5 ml PRRSV and 1.0 ml PPV intranasally by slowly dripping each inoculum into both nostrils.

2.5. Laboratory testing

2.5.1. Serology. All serum samples were tested by an ORF2-based anti-PCV2 IgG ELISA (Nawagitgul et al., 2002). Samples were considered positive if the calculated sample-to-positive (S/P) ratio was equal to or less than 0.2 (Nawagitgul et al., 2002). All serum samples collected at D28 and D49 were also tested for the presence of specific antibodies to PRRSV and PPV by a commercial ELISA (Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit, HerdChek* PRRS 2XR; IDEXX Laboratories Inc., Westbrook, MA, USA), in which S/P ratios equal to or less than 0.40 were considered negative and a hemagglutination inhibition assay as described (Mengeling et al. 1988), respectively.

2.5.2. Molecular testing. Viral DNA and RNA was extracted from the serum samples and swab suspensions after vigorous stirring using the MagMax^{TM} Viral RNA Isolation Kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) on the KingFisher Flex System (ThermoFisher Scientific, Pittsburgh, PA, USA). All serum samples and swabs collected on D0,D7, D14, D21, D28, D35, D42, and D49 were tested for presence and amount of PCV1-2 and all serum samples and swabs collected on D28, D35, D42, and D49 were tested for presence and amount of PCV2 DNA, PRRSV RNA, and PPV DNA. The presence and amount of PCV2 DNA was detected by a quantitative real-time PCR assay as described (Opriessnig et al., 2003) except that a 25 µl total reaction volume and 2.5 µL of DNA extract were used in this study. The PCV2 real-time PCR targets PCV2-ORF1 which is only present in the challenge virus, and the PCV1-2 PCR targets an overlapping region of ORF1 from PCV1 and ORF2 from PCV2 which is only present in the vaccine virus but not in the challenge virus. The combination of the results obtained with the two PCR assays allowed differentiating vaccine virus (PCV2 PCR negative but PCV1-2 PCR positive) from challenge virus (PCV2 PCR positive but PCV1-2 PCR negative).All serum samples that were positive for PCV2 DNA at D49 were further tested with a PCV2a/2b differential real-time PCR assay as described (Opriessnig 2010) in order to determine the PCV2 genotype(s) present. In addition, quantitative real-time PCR assay for PPV DNA and PRRSV RNA were performed on D49 serum samples as described (Opriessnig et al., 2011b). Viral concentrations were expressed as the mean viral DNA or RNA copy number per ml of serum. Samples with no signal by a cycle-threshold (C_T) of 40 were considered negative.

2.6. Postmortem testing

2.6.1. Necropsy. All pigs were euthanized by intravenous pentobarbital sodium (Fatal-Plus; Vortech Pharmaceutical, LTD, Dearborn, MI, USA) overdose and on D49 and a necropsy was performed.

2.6.2. Macroscopic lesions. The total amount of macroscopic lung lesions ranging from 0% to 100% was estimated and scored as described (Halbur et al., 1995a). All the scores were estimated in a blinded fashion to the treatment status. Sections of the mediastinal, mesenteric, superficial inguinal, external iliac and tracheobronchial lymph nodes, spleen, tonsil and lungs were collected during necropsy and fixed in 10% neutral-buffered formalin and routinely processes for histopathology

2.6.3. Microscopic lesions. Lung tissues sections were scored for the presence and severity of interstitial pneumonia, with scores ranging from 0 (normal) to 6 (severe diffuse) (Halbur et al., 1995b). Lymphoid tissue, including lymph nodes, tonsil, and spleen were also evaluated for lymphoid depletion and histiocytic replacement of follicles with scores ranging from 0 (none) and 3 (severe) (Opriessnig et al., 2004). The overall microscopic lymphoid lesions score which consists of lymphoid depletion, histiocytic inflammation and PCV2-antigen present in lymphoid tissues was calculated as previously described and ranged from 0 (normal) to 9 (severe) (Opriessnig et al., 2004). Briefly, the scores (lesions and PCV2-immunohistochemistry or IHC) of seven lymphoid tissues ([lymph node pool] × 5, spleen, and tonsil) were added up and divided by 7. The lymph node pool consisted of mediastinal, mesenteric, superficial inguinal, external iliac and tracheobronchiolar lymph nodes. The mean treatment group lymphoid score was calculated and compared between groups. Pigs were grouped into four categories based on overall microscopic lymphoid lesion scores: 0 (normal), 1-3 (mild), 4-6 (moderate), 7-9 (severe) (Opriessnig et al., 2004).

2.6.4. Immunohistochemistry (IHC). Detection of PCV2 antigen was performed by PCV2 IHC on selected formalin-fixed and paraffin-embedded sections of lymph nodes, spleen and tonsil using a rabbit polyclonal antiserum as previously described (Sorden et al., 1999). The PCV2 antigen scores ranged from 0 (negative) to 3 (more than 50% of the lymphoid follicles contain cells with PCV2 antigen staining). Scoring was done by a veterinary pathologist (TO) blinded to treatment status (Opriessnig et al., 2004).

2.7. Statistical analysis

For data analysis, SAS® software version 9.2.0 (SAS Institute, Cary, NC, USA) was used. Summary statistics were calculated for groups to assess the distributional property and data that were not distributed normally (PCR data) were log transformed prior to analysis. A constant number (1) was added to each number in the data set prior to log transformation. The model to analyze continuous data collected over time (log transformed PCV2, PRRSV and PPV genomic copies, and ELISA S/P ratios) was a repeated measures analysis of variance (ANOVA), where group, time and their interaction were fixed effects and pig was considered as random effect. Differences among the interacting groups in the repeated measures ANOVA or the one-way ANOVA were assessed using Tukey's t-test. A p-value of less than 0.05 was considered significant. Percent reduction for PCR data was measured as follows: 100− (100×mean log10 genomic copies/ml in the vaccinated group or PCV2a inoculated group)/(mean log10 genomic copies/ml in positive control animals or PCV2a no inoculated group). Non-parametric Kruskal-Wallis ANOVA was used on non-repeated measures of data (histopathological and IHC scoring), and if significant, Wilcoxon tests were used to evaluate differences between pairs. Differences in prevalence and incidence were determined by using Fisher's exact test.

Results

3.1. Clinical disease and average daily weight gain

Clinical signs were not evident in any of the pigs after vaccination until challenge at D28. After challenge, most of the inoculated pigs developed mild respiratory disease characterized by occasional sneezing and increased respiratory rates. One VAC/2a-Ch/2b-PRRSV-PPV pig which was poor doing since arrival developed non-specific bloody diarrhea and was euthanized on D34. One Ch/PRRSV-PPV pig died after routine bleeding on D42 due to massive hemorrhages in the neck area. Finally, on D48 1/8 Ch/2a-2b-PRRSV-PPV pigs developed severe respiratory distress, cyanosis, and was unable to stand and had to be euthanized.

From the time of vaccination to challenge between D0 and D28, the average daily gain was not different ($p=0.9039$) between non-vaccinated pigs (363.6 g ±27.9 g) or pigs vaccinated with PCV1-2a (342.2 g ±44.9 g) or PCV1-2b (347.0 g ±47.3 g). Similarly, there was no significant difference in average daily gain after challenge until necropsy between D28 and D49 (data not shown).

3.2. Serology

3.2.1. Anti-PCV2 response. All pigs were seronegative for PCV2 at D0 and negative controls and Ch/PRRSV-PPV pigs remained seronegative until D49. Vaccinated pigs started to seroconvert against PCV2 by D14 (14 days after vaccination) and non-vaccinated PCV2 challenged pigs started to seroconvert to PCV2 by D35 (7 days after challenge). Pigs vaccinated with the PCV1-2b vaccine had a stronger and faster anti-PCV2 IgG response compared to pigs vaccinated with the PCV1-2a vaccine. On D14, 81.3% (13/16) of the pigs vaccinated with PCV1-2 were seropositive whereas only 31.3% (5/16) of the pigs vaccinated with PCV1-2a had seroconverted to PCV2. PCV1-2b vaccinated pigs had significantly ($p<0.05$) higher mean group S/P ratios compared to PCV1-2a vaccinated pigs at D14, D21, and D28. Interestingly, the Ch/2a-b-PRRSV-PPV pig that had to be euthanized on D48 had not seroconverted to PCV2.

3.2.2 PPV and PRRSV serology. All pigs were negative for PRRSV specific anti-IgG at D28 and the majority (94.4%; 51/54) of the pigs challenged with PRRSV had seroconverted by D49 (data not shown). The negative control pigs had no evidence of anti-PRRSV antibodies by D49. As expected, the pigs were positive for PPV antibodies at D28 and remained seropositive until D49. Pigs challenged with PPV had higher titers at D49 compared to the negative control pigs (data not shown).

3.3. Viremia and virus shedding

3.3.1. Chimeric vaccine virus. Chimeric PCV2 viremia was detected in 10/16 pigs vaccinated with PCV1-2a and in 16/16 pigs vaccinated with PCV1-2b. Whereas PCV1-2b vaccinated pigs were viremic for 2 to 7 consecutive weeks, PCV1-2a viremia was sporadic and often limited to one positive detection day. Pigs vaccinated with PCV1-2b had significantly ($p<0.05$) higher concentrations of PCV1-2 DNA in serum on D7, D14, D21, D28, D35 and D49. PCV1-2 DNA shedding through nasal secretions and feces mimicked the PCV1-2 serum profiles for the two vaccinated groups.

3.3.2. PCV2. PCV2 DNA was not detected in any of the non-PCV2 infected control groups (negative controls, Ch/PRRSV-PPV) (data not shown). In contrast, all non-vaccinated PCV2 challenged pigs (Ch/2b-PRRSV-PPV, Ch/2a-2b-PRRSV-PPV) were viremic on D35, D42 and D49. At D49, the percentage of PCV2 viremia reduction was 25% (VAC2a-Ch/2b-PRRSV-PPV), 44.1% (VAC2a-Ch/2a-2b-PRRSV-PPV), or 100% (VAC2b-Ch/2b-PRRSV-PPV and VAC2b-Ch/2a-b-PRRSV-PPV). The predominant PCV2 genotype present at D49 was determined by differential PCV2a/2b real-time PCR. Only PCV2b DNA and not PCV2a DNA was identified in groups challenged with PCV2b alone (VAC/2a-Ch/2b-PRRSV-PPV and Ch/2b-PRRSV-PPV) which indicates presence of challenge virus. PCV2b DNA in addition to PCV1-2 DNA was identified in VAC/2b-Ch/2a-2b-PRRSV-PPV pigs challenged with both PCV2a and PCVb and corresponded to vaccine virus as the pigs were negative for ORF1-PCV2 DNA. PCV2a DNA (4/8 pigs) and PCV2b DNA (5/8 pigs) were identified in 5/8 VAC/2a-Ch/2a-2b-PRRSV-PPV pigs which indicated a mixed population of both challenge viruses in 4/8 pigs in this group. Finally, high concentrations of PCV2a and PCV2b DNA were detected in 8/8 Ch/2a-2b-PRRSV-PPV pigs indicating a mixed population of both challenge viruses in all pigs in this group replicating at similar high levels.

3.3.3. PPV and PRRSV. The prevalence rates and concentrations of PRRSV RNA and PPV DNA at D49 in the different groups are summarized in Table 1. There were no significant differences among groups.

3.4. Macroscopic lesions

The pig that was euthanized on D34 (6 days after PCV2b-PRRSV-PPV challenge) had no recognizable macroscopic lung lesions while the pig that died during bleeding on D42 (14 days after PRRSV-PPV challenge) had a lung lesion score of 78. The pig that died on D48 (20 days after PCV2-PCV2b-PRRSV-PPV challenge) due to severe respiratory distress had a lung lesion score of 93% and the lung lesions were characterized by

diffuse severe interlobular edema, failure to collapse, and 93% of the lung surface were dark-red purple and consolidated. At D49, macroscopic lung lesions were characterized by multifocal to diffuse discoloration (mottle-tan), failure to collapse, and cranioventral purple-tan consolidations. The mean group lung lesions scores are summarized in Table 2. When group mean lung lesions scores of non-vaccinated and PCV2-challenged pigs were compared to vaccinated and PCV2-challenged pigs, the scores were significantly ($p=0.0003$) higher in non-vaccinated pigs (n=16; 40.8±6.1) compared to pigs vaccinated with PCV1-2a (n=15; 15.9±3.4) or pigs vaccinated with PCV1-2b (n=16; 18.4±11.0).

3.5. Microscopic lesions and amount of PCV2 antigen

The pig that was euthanized on D34 (6 days after PCV2b-PRRSV-PPV challenge) had a microscopic interstitial pneumonia score of 1 and the pig that died during bleeding on D42 (14 days after PRRSV-PPV challenge) had focal moderate interstitial pneumonia (microscopic interstitial pneumonia score of 3). Microscopic lung lesions for all other pigs are summarized in Table 2. Individual pigs in non-vaccinated groups (two each in the Ch/2b-PRRSV-PPV group and the Ch/2a-2b-PRRSV-PPV group) had severe diffuse interstitial pneumonia (score=6) which was multifocally granulomatous (including the pig that had to be euthanized on D48).

The two pigs that died at D34 or D42 had no microscopic lesions in lymphoid tissues. While the majority of the pigs had normal lymphoid tissues or developed only mild lesions, moderate lesions (score range from 4-6) were observed in 7/8 Ch/2b-PRRSV-PPVpigs, 2/8 VAC/2a-Ch/2a-2b-PRRSV-PPV, 1/8 VAC/2b-Ch/2a-2b-PRRSV-PPV, and 3/8 Ch/2a-2b-PRRSV-PPV pigs. Overall, 4 pigs developed systemic lymphoid lesions associated with abundant PCV2 antigen consistent with PCVAD (overall lymphoid lesions scores between 7 and 9) under the study conditions. All 4 pigs belonged to the Ch/2a-2b-PRRSV-PPV group. PCV2 antigen was not detected in any of the pigs not challenged with PCV2 (negative controls or Ch/PRRSV-PPV). The PCV2 antigen prevalence (positivePCV2 IHC staining in at least one lymphoid tissue) for the remaining groups was as follows: 0/7 for VAC/2a-Ch/2b-PRRSV-PPV; 1/8 for VAC/2b-Ch/2b-PRRSV-PPV; 8/8 for Ch/2b-PRRSV-PPV; 2/8 for VAC/2a-Ch/2a-2b-PRRSV-PPV; 3/8 for VAC/2b-Ch/2a-2b-PRRSV-PPV; and 8/8 for Ch/2a-2b-PRRSV-PPV.

Discussion

The PCV2b genotype, first recognized in 2006 (Cheung et al., 2007; Gagnon et al., 2007), now predominates in the global pig population (Patterson and Opriessnig, 2010). However, the commercially available PCV2 vaccines in the U.S. are all based on the PCV2a genotype. Cross-protection between PCV2 genotypes has been demonstrated experimentally (Opriessnig et al., 2007b; Fort et al., 2008) and this is further supported by the excellent efficacy of PCV2a based vaccines under field conditions (Kixmöller et al., 2008; Horlen et al., 2008; Fraile et al., 2012), yet PCVAD outbreaks in well vaccinated herds do occur. Recently, PCV2b but not PCV2a was identified in serum from vaccinated healthy growing pigs in a survey conducted in the USA (Shen et al., 2012). Moreover, during 2010 the PCV2 genotypes in plasma samples obtained from regional Midwest slaughter houses were investigated and PCV2b DNA was found in a high percentage of the samples in contrast to PCV2a (Shen et al., 2011). Similarly, in a field study analyzing PCV2 genotypes in five breeding herds across North America, PCV2b and not PCV2a was highly prevalent (Shen et al., 2010a). These findings indicate that PCV2a vaccines, although they generally prevent clinical disease and reduce PCV2a prevalence, they do not necessarily reduce the prevalence of PCV2b indicating perhaps less than ideal cross-protection. The objective of this study was to compare two PCV2 vaccines based on 2a and 2b, respectively, side by side in the growing pig challenge model.

Under the study conditions, PCV2 vaccination using a live-attenuated chimeric vaccine resulted in recognizable and increasing concentrations of PCV1-2 viremia in a portion of the vaccinated pigs. Vaccine induced clinical signs were not observed under the study conditions and after vaccination and before challenge the average daily gain was comparable to that of non-vaccinated pigs. Around 21 days after vaccination, viremia levels of vaccine virus peaked and virus concentrations declined thereafter. Seroconversion to PCV2 in vaccinated pigs was detectable by D14. Interestingly, vaccination with PCV1-2b resulted in significantly higher

levels of PCV1-2 viremia that was of longer duration in addition to higher PCV1-2 concentrations shed in feces and nasal secretions compared to what was observed in pigs vaccinated with PCV1-2a. Despite (or because of) higher PCV1-2 DNA levels in serum and feces and nasal secretions, pigs vaccinated with PCV1-2b had a more robust humoral immune response as evidenced by significantly higher ELISA S/P ratios compared to pigs vaccinated with PCV1-2a. The number of infectious viruses present in each of two vaccines was determined by titration in PK-15 cells and both vaccines had similar $TCID_{50}$ levels at the time of vaccination.

Vaccine-induced protection was measured by several parameters including reduction of PCV2 viremia after challenge. While both vaccines significantly reduced PCV2 viremia in vaccinated and challenged pigs, there was a significant different between the vaccine types (2a or 2b based). After challenge, pigs vaccinated with PCV1-2b had significantly lower concentrations of PCV2 DNA in serum compared to pigs vaccinated with PCV1-2a. Furthermore, PCV2 viremia was not detectable in PCV2b vaccinated pigs 14 (D42) or 21 (D49) days after challenge. The percentage of viremia reduction on D49 compared to non-vaccinated positive control pigs ranged between 25-44.1% for PCV1-2a vaccinated pigs while it was 100% for pigs vaccinated with PCV1-2b. However, significant differences in presence or severity of microscopic lesions were not observed between vaccinated pigs regardless of the PCV2 genotype present in the vaccine.

Recent epidemiologic investigations revealed a high frequency of concurrent PCV2a and PCV2b under field conditions in certain countries (Hesse et al., 2008; Shen et al., 2010a; Shen et al., 2011), and a possible role of combined PCV2a/PCV2b infection in triggering PCVAD has been suggested (Zhai et al., 2011). PCV2a, PCV2b or both PCV2a and PCV2b, were detected in 43.3% (42/97), 31.9% (31/97) and 24.8% (24/97) of investigated U.S. serum samples, respectively (Hesse et al., 2008). In further support of this, 11.9% of serum samples collected from healthy sows in North America in 2009 were positive for PCV2a and PCV2b DNA (Shen et al., 2010a). More recently, concurrent PCV2a and PCV2b infection was identified in 31.6% (12/38) of clinically affected pigs, but not in healthy pigs (Zhai et al., 2011). Furthermore, high frequencies of PCV2a-PCV2b coinfection were identified in tissues from subclinical or clinically-affected pigs in Switzerland (Khaiseb et al., 2011). In infected cells with replicating virus both genotypes were present leading to the authors to suggest that simultaneous co-replication of PCV2a and PCV2b may be a requirement for obtaining high viral loads and subsequent disease expression (Khaiseb et al., 2011).

While no differences in weight gain were determinable after challenge, one Ch/2a-2b-PRRSV-PPV pig developed severe respiratory distress and had to be euthanized on D48. PCV2a-2b coinfected pigs had significantly more severe lung lesions compared to pigs infected with 2b alone. In this study, concurrent PRRSV and PPV infection was used in all pigs except negative controls to better mimic field conditions. Although PCV2 is the primary and essential etiological agent in the pathogenesis of PCVAD, experimental and field studies showed that other pathogens are necessary to trigger PCVAD (Segalés, 2011; Opriessnig et al., 2012). Infection of pigs with PRRSV and PPV and without PCV2 resulted in mild lung lesions in most pigs further highlighting the role and importance of PCV2 in the porcine respiratory disease complex. After microscopic examination, the clinically affected pig and three other pigs in the non-vaccinated 2a-2b coinfected group had severe systemic lymphoid lesions compatible with PCVAD. In contrast, the severity of microscopic lymphoid lesions was moderate in most of the Ch/2b-PRRSV-PPV pigs indicating an effect (additive or potentiating) of concurrent PCV2a infection on development of lesions. In further support of this, in the gnotobiotic pig model three days old pigs inoculated with PCV2a and subsequently infected with PCV2b developed severe disease (Harding et al., 2010).

Under the study conditions, vaccination with an experimental attenuated-live chimeric PCV2 vaccine based on the 2b genotype was more effective in protecting pigs against the effects of PCV2b or concurrent PCV2a-PCV2b challenge compared to a vaccine based on the PCV2a genotype. In non-vaccinated pigs, coinfection with PCV2a and PCV2b resulted in clinical manifest PCVAD and enhanced systemic lymphoid lesions in conventional pigs compared to challenge with PCV2b alone.

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