RESEARCH REPORT
SWINE HEALTH

Title: Comparison of the level of protection and long-term duration of immunity induced by different commercial PCV2 vaccines and an experimental PCV1-2 live vaccine in conventional pigs – **NPB #08-269**

Investigator: Tanja Opriessnig, Dr med vet, PhD

Institution: Iowa State University

Date Submitted: March 1, 2010

Industry Summary:

The efficacy of commercial porcine circovirus type 2 (PCV2) vaccines and a live PCV1-2a chimeric vaccine were compared in conventional PCV2 positive piglets using a PCV2-PRRSV-PPV coinfection model. Seventy-three 2-week-old pigs were randomized into seven groups (PCV1-2, FDAH-1, BIVI-1, Intervet-2, FDAH-2, Positive and Negative) based on vaccine given and dose size; also included were a positive and negative control. Pigs in the vaccinated groups were vaccinated at 3 weeks of age (one dose) or at 3 and 6 weeks (two dose). Pigs in the positive and negative groups received no vaccination. At 16 weeks of age all pigs excluding negative controls were challenged with PRRSV, PPV and PCV2b. All the pigs except those in the negative control group were viremic for PRRSV and PPV at 7 days post inoculation (dpi). At 14-21 dpi, 100% pigs were viremic for PRRSV and 45-100% were viremic for PPV in the positive control group, whereas the viremia percentage in vaccine groups was 90-100% for PRRSV and 64-100% for PPV. Blood was collected on a weekly basis and tested for anti- PCV2 antibodies using an ELISA and for the presence of PCV2 DNA by quantitative real-time PCR. There were no significant differences in the mean group PCV2 ELISA S/P ratios between one-dose and two-dose vaccination regimens. All vaccinated groups had significantly ($p<0.05$) lower prevalence of PCV2 viremia and mean $\log_{10}$ PCV2 loads at 16 weeks compared to the positive control group, with an overall reduction of PCV2 viremia by 49.9-89.5%, specifically78.9% for one-dose vaccines and 68.1% for two-dose vaccines. Pigs were necropsied three weeks after challenge (21 dpi) corresponding to 19 weeks of age. Microscopic lesions, characterized by mild interstitial pneumonia and mild lymphoid depletion and histiocytic replacement in lymphoid tissues, were present in all challenged groups. There were no significant differences in mean group scores for any of the evaluated lesions among challenged groups. In general, vaccine regimens were effective in reducing natural occurring PCV2 viremia at 16 weeks of age and after PCV2 challenge, demonstrating the capability of the products to induce a lasting protective immunity despite presence of PCV2 viremia at vaccination.

Contact Information: tanjaopr@iastate.edu

Scientific Abstract
The efficacy of commercial porcine circovirus type 2 (PCV2) vaccines and a live PCV1-2a chimeric vaccine were compared in conventional PCV2 positive piglets using a PCV2-PRRSV-PPV coinfection model. Seventy-three 2-week-old pigs were randomized into seven groups (PCV1-2, FDAH-1, BIVI-1, Intervet-2, FDAH-2, Positive and Negative) based on vaccine given and dose size; also included were a positive and negative control. Pigs in the vaccinated groups were vaccinated at 3 weeks of age (one dose) or at 3 and 6 weeks (two dose). Pigs in the positive and negative groups received no vaccination. At 16 weeks of age all pigs excluding negative controls were challenged with PRRSV, PPV and PCV2b. All the pigs except those in the negative control group were viremic for PRRSV and PPV at 7 days post inoculation (dpi). At 14-21 dpi, 100% pigs were viremic for PRRSV and 45-100% were viremic for PPV in the positive control group, whereas the viremia percentage in vaccine groups was 90-100% for PRRSV and 64-100% for PPV. Blood was collected on a weekly basis and tested for anti- PCV2 antibodies using an ELISA and for the presence of PCV2 DNA by quantitative real-time PCR. There were no significant differences in the mean group PCV2 ELISA S/P ratios between one-dose and two-dose vaccination regimens. All vaccinated groups had significantly ($p<0.05$) lower prevalence of PCV2 viremia and mean $\log_{10}$ PCV2 loads at 16 weeks compared to the positive control group, with an overall reduction of PCV2 viremia by 49.9-89.5%, specifically78.9% for one-dose vaccines and 68.1% for two-dose vaccines. Pigs were necropsied three weeks after challenge (21 dpi) corresponding to 19 weeks of age. Microscopic lesions, characterized by mild interstitial pneumonia and mild lymphoid depletion and histiocytic replacement in lymphoid tissues, were present in all challenged groups. There were no significant differences in mean group scores for any of the evaluated lesions among challenged groups. In general, vaccine regimens were effective in reducing natural occurring PCV2 viremia at 16 weeks of age and after PCV2 challenge, demonstrating the capability of the products to induce a lasting protective immunity despite presence of PCV2 viremia at vaccination.

Introduction

Porcine circovirus (PCV) is a circular single-stranded DNA virus in the *Circoviridae* family [1]. PCV was first isolated by Tischer et al. in 1974 in the porcine kidney cell line (PK-15) ATCC CCL-33 [2]. This type of PCV was found to be non-pathogenic to pigs [3]. In 1991, a disease termed postweaning multisystemic wasting syndrome (PMWS) was observed in Canada, and associated with a genomically distinct type of PCV [4, 5]. Since the pathogenic and nonpathogenic PCVs were different in terms of DNA sequence and antigenicity, virus isolated from the PK-15 cells was designated PCV1 and the virus isolated from pigs with PMWS, PCV2 [6]. Now it is generally accepted that PCV2 is associated with a number of disease manifestations collectively known as porcine circovirus–associated disease (PCVAD), which includes systemic disease or PMWS, porcine respiratory disease complex (PRDC), porcine dermatitis and nephropathy syndrome (PDNS), pneumonia, diarrhea in grow-finish pigs, and reproductive failure [7].

Although PCV2 is a necessary pathogen in the development of PCVAD, other factors seem to be needed for full development of the syndrome. The severity of the lesions in experimental infections was substantially enhanced if pigs were co-infected with PCV2 and porcine parvovirus (PPV) or porcine reproductive and respiratory syndrome virus (PRRSV). When 1-day-old gnotobiotic pigs [8] or colostrum-deprived (CD) pigs [9, 10] were inoculated with PCV2 and PPV, a higher incidence of clinical disease, more severe microscopic lesions and more widely distributed PCV2 antigen were observed compared to pigs inoculated with PCV2 alone. Similarly, when 1-to-2-day-old CD pigs [11] or 5-week-old PCV2 seropositive pigs [12] were experimentally infected with PCV2 and PRRSV, enhanced replication and distribution of PCV2 as well as severe clinical disease and lesions were observed compared to pigs inoculated with PCV2 alone. These experimental results have been further supported by epidemiological data, and PRRSV and PPV have been observed in PCVAD affected farms. PRRSV was detected in 51.9% of the PMWS cases in a retrospective investigation [13]. Immunohistochemical, serological and molecular studies revealed that PRRSV was present on a farm during outbreaks of PMWS [14, 15] and in various tissues associated with PDNS cases [16]. The odds of PMWS were increased when nursery pigs tested positive for PPV and PRRSV [17] and if the animals were concurrently infected with PCV2 and PRRSV [18]. Tissue homogenates used to reproduce the lesions of

PMWS in neonatal gnotobiotic piglets were found to contain both PCV2 and PPV [19]. PPV and PCV2 were detected in 12/69 cases of PMWS by immunohistochemistry (IHC) and polymerase chain reaction (PCR) [6]. These results revealed that coinfections with PRRSV or PPV may be important cofactors in the pathogenesis of PCVAD.

It has been shown that vaccination is an effective tool to reduce PCVAD losses in swine populations. Currently there are different types of antigens available in commercial PCV2 vaccines, including inactivated PCV2 virus, killed chimeric PCV1-2 virus, and ORF2 protein expressed in the baculovirus system [7]. Under experimental conditions, the efficacy of PCV2 commercial vaccines has been evaluated in PCV2 naïve pigs (free of PCV2 virus and antibody) or PCV2 negative pigs (free of PCV2 virus). It was found that PCV2 vaccination induces neutralizing antibodies and reduces PCV2 viremia and associated lesions in naïve pigs [20, 21], and in PCV2 negative piglets with passively-acquired anti-PCV2 IgG antibodies. [22]. Under field conditions, the efficacy of commercial PCV2 vaccines has been evaluated in farms suffering from PCVAD [23-27]; in vaccinated populations, mortality and cull rates were reduced and the average daily weight gain (ADWG) was improved.

Most pigs in the field become infected with PCV2 early in life and PCV2 DNA is frequently detected in serum samples obtained from 3-week-old pigs (T. Opriessnig; personal observation). Until now experimental investigations of PCV2 vaccine efficacy were confined to PCV2 naïve or negative pigs, and no data is available regarding the effect and efficacy of vaccines in PCV2 positive pigs under experimental condition. The objectives of this study were to investigate the efficacy of three commercial vaccines and one experimental PCV1-2a live vaccine in conventional PCV2 positive (PCV2 viremic and antibody positive) pigs in a PCV2-PRRSV-PPV co-infection model.

Objectives

1) Determine and compare the duration of immunity of commercially available PCV2 vaccines (FDAH, BIVI, Intervet) and an experimental live PCV2 vaccine in conventional pigs (PCV2 antibody positive).
2) Determine whether one dose (FDAH-1, BIVI-1) and two dose PCV2 (Intervet-2, FDAH-2) vaccine products provide equivalent long-term protection in conventional growing pigs.
3) Determine if there is a benefit of using a live PCV2 vaccine versus a killed product in piglets with passively acquired antibodies.

Materials and Methods

2.1 Animals and housing

Colostrum-fed, cross-bred conventional pigs were purchased from a herd confirmed to be free of PRRSV, PPV and swine influenza virus (SIV) by routine serology. The pigs were weaned at 2 weeks of age and transported to the Livestock Infectious Disease Isolation Facility at Iowa State University, Ames, Iowa. All pigs except the negative control group and the PCV1-2 group were randomly assigned to one of two rooms each containing six 2.5 m × 3.6 m raised wire decks equipped with one nipple drinker and one self-feeder regardless of vaccination status. The negative control group was housed separately in a similar room. To avoid potential spread of the live vaccine to other treatment groups, the PCV1-2 group was also housed in a separate room of similar design. The pigs were fed with a phased diet for growing pigs free of animal proteins and antibiotics.

2.2 Experimental design

Seventy-three 2-week-old, conventional piglets were randomly divided into seven groups, including five vaccine groups and two control groups (Table 1).

Table 1: Study design.

Group	N	Vaccine	Dose	Vaccination		Challenge
				3 weeks	6 weeks	16 weeks
PCV1-2	11	Live PCV1-2 orally		2 ml	-	Yes
FDAH-1	11	Suvaxyn® PCV2	One-dose	2 ml	-	Yes
BIVI-1	11	Ingelvac® CircoFLEX™	One-dose	1 ml	-	Yes
Intervet-2	10	Circumvent® PCV2	Two-dose	2 ml	2 ml	Yes
FDAH-2	10	Suvaxyn® PCV2	Two-dose	1 ml	1 ml	Yes
Positive	11	-	-	-	-	Yes
Negative	9	-	-	-	-	No

Pigs were vaccinated at 3 weeks of age for one-dose vaccines and PCV1-2 live vaccine, and 3 and 6 weeks of age for two-dose vaccines according to Table 1. At 16 weeks of age, pigs in all groups except the negative control group were challenged with PCV2b, PRRSV and PPV. Blood was collected on a weekly basis for serology and molecular analysis. Three weeks after challenge (19 weeks of age), all pigs were necropsied and macroscopic and microscopic lesions were compared among groups. The amount of PCV2 antigen in lymphoid tissues was determined by immunohistochemistry (IHC) [28].

2.3 Vaccination

Three commercial PCV2 vaccines and one live PCV1-2a vaccine were used in this study (Table 1). Vaccination was done for all pigs in a similar way: each pig received the amount of vaccine as prescribed by the manufacturer, which was given intramuscularly into the right side of the neck. Pigs in the FDAH-1 group were vaccinated once with 2 ml Suvaxyn® PCV2 (Fort Dodge Animal Health Inc.; serial number 1861161A), pigs in the FDAH-2 group were vaccinated twice with 1 ml of Suvaxyn® PCV2, pigs in the BIVI-1 group were vaccinated once with 1ml of Ingelvac® CircoFLEX™ (Boehringer Ingelheim Vetmedica Inc.; serial number 309-009B) and pigs in the Intervet-2 group were vaccinated twice with 2 ml of Circumvent™ PCV2 (Intervet Inc., Millsboro, Denver, USA; serial number 02137920). PCV1-2 pigs received 1 ml of a live PCV1-2 chimeric vaccine based on PCV2a isolate 40895 [29] at a dose of $10^{4.0}$ $TCID_{50}$/ml.

2.4. Challenge

At 16 weeks of age, each pig in the challenged groups (Table 1) was challenged intranasally with 1.5 ml PCV2b, 2.5 ml PRRSV and 1 ml PPV inoculum. PCV2b isolate NC-16845 was propagated on PK-15 cells to get an inoculum with a 50% tissue culture infective doses ($TCID_{50}$) of $10^{4.5}$ $TCID_{50}$ per ml. PRRSV isolate ATCC VR2385 was propagated on MARC-145 cells and the inoculum had a titer of $1\times10^{5.0}$ $TCID_{50}$/ml. The PPV challenge stock was originated from a tissue homogenate and the final inoculum had a titer of $10^{6.0}$ $TCID_{50}$/ml.

2.5. Serology

Blood samples were collected upon arrival of the pigs at 2 weeks of age, then weekly from 6 weeks of age until necropsy at 19 weeks of age. The serum samples collected at 2, 6, 16 and 19 weeks of age were tested by a ORF2-PCV2 IgG ELISA [30]. 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. In addition, serum samples from all pigs at 16 and 19 weeks of age were tested for the presence of anti-PRRSV antibodies by ELISA (HerdChek PRRS virus antibody test kit 2XR, IDEXX Laboratories Inc., Westbrook, MA, USA), and for the presence of antibodies to PPV by hemagglutination inhibition (HI) assay [31].

2.6. Clinical evaluation

All pigs were weighed on the day of arrival and weekly thereafter until 19 weeks of age. Following coinfection with PCV2, PRRSV and PCV2 at 16 weeks of age, the pigs were monitored and scored daily for clinical signs including sneezing, lethargy, and coughing [32].

2.7. PCV2, PRRSV and PPV viremia detection

All the serum samples (week 2, 6-19) were tested for PCV2 DNA loads, and samples collected after challenge (week 17-19) were also tested for the presence and amount of PRRSV RNA and PPV DNA. Viral DNA and RNA were extracted from serum samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) and the QIAamp Viral RNA Mini Kit (Qiagen) respectively. The amount of PCV2 DNA was detected by a quantitative real-time PCR [33], and the amount of PRRSV RNA was detected by a quantitative real-time RT-PCR method [34]. Viral concentrations were expressed as the mean viral DNA or RNA copy numbers per ml of serum.

PPV DNA was detected by a quantitative real-time PCR which were designed according to the VP2-coding region of PPV. The real-time PCR reaction consisted of a total volume of 25 µl containing 12.5 µl of the commercially available master mix (TaqMan® Universal PCR master mix, PE Applied Biosystems), 2.5 µl DNA, 0.4 µM of each primer, and 0.2 µM of probe. The reaction was carried out in a 7500 Fast Real-Time PCR system (ABI, Foster City, CA) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Serial dilutions of a recombinant plasmid containing PPV VP2 gene were used for obtaining a standard curve.

2.8. PCV1-2 PCR

A forward primer,a reverse primer and a probe were designed in the region spanning PCV2 ORF2 (Genbank accession no. AF264042) and PCV1 ORF1 (Genbank accession no. U49186). The real-time PCR reaction consisted of a total volume of 25 µl containing 12.5 µl of TaqMan Universal PCR master mix (Applied Biosystems), 1 µl of each forward and reverse primer (final concentration 0.4 µM), 0.5 µl TaqMan probe (final concentration 0.2 µM), 7.5 µl nuclease-free water and 2.5 µl extracted DNA. The PCR cycling parameters were 2 min at 50° C, 10 min at 95° C, followed by 40 cycles of 15 sec at 95° C, 30 sec at 55° C and 1 min at 60° C. Five progressive 10-fold dilutions of a PCV1-2 chimeric DNA clone were used to generate a standard curve.

2.9. Necropsy

All pigs were humanely euthanized by phenobarbital overdose and necropsied at 19 weeks of age. The total extent of macroscopic lung lesions (ranging from 0 to 100%) was estimated and scored [35]. Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsil, thymus, ileum, kidney, colon, spleen, and liver were collected at necropsy, fixed in 10% neutral-buffered formalin, and routinely processed for histological examination.

2.10. Histopathology

Microscopic lesions were evaluated by a pathologist (TO) blinded to group designations. Lung sections were scored for the presence and severity of interstitial pneumonia, ranging from 0 (normal) to 6 (severe diffuse) [32]. Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 (none) to 3 (severe). The overall microscopic lymphoid lesion score, which accounts for lymphoid depletion and histiocytic inflammation, was calculated as previously described [36].

2.11. Immunohistochemistry

Immunohistochemistry (IHC) for detection of PCV2-specific antigen was performed on selected formalin-fixed and paraffin-embedded sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsil, spleen, and thymus using a rabbit polyclonal antiserum [28]. PCV2 antigen scoring was done by a pathologist (TO) blinded to treatment groups. Scores ranged from 0 (no signal) to 3 (more than 50% of lymphoid follicles contained cells with PCV2 antigen staining) [36]. The mean group score was determined for each tissue and compared among groups.

2.12. Statistical analysis

Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) using the JMP® software version 8.0.1 (SAS Institute, Cary, NC). Student's t-test was used for each pair comparisons; a p-value of less than 0.05 was set as a statistically significant level throughout this study. Real-time PCR results (copies per ml of serum) were $\log_{10}$ transformed prior to statistical analysis. A chi-square test was used to analyze the significance of incidence. Percent reduction for PCR data was measured as follows: $100 - [(100 \times \text{mean } \log_{10} \text{ genomic copies/ml in the vaccinated group}) \div (\text{mean } \log_{10} \text{ genomic copies/ml in positive control animals})]$.

Results

3.1. Clinical presentation

Clinical disease was not observed in the non-challenged animals, whereas mild respiratory symptoms including mild sneezing and nasal discharge were observed in all the challenged pigs with no significant differences among groups. The ADWG ranged from 1.35-1.49 lb/day during growing period (2-16 weeks of age) with no significant differences among groups, whereas after challenge, 4 of 5 vaccine groups (PCV1-2, BIVI-1, Intervet-2, FDAH-2) had significantly ($p<0.05$) higher ADWG compared to the positive control group. There were no significant differences in ADWG between one-dose and two-dose vaccination groups.

3.2. Anti-PCV2-IgG antibody levels

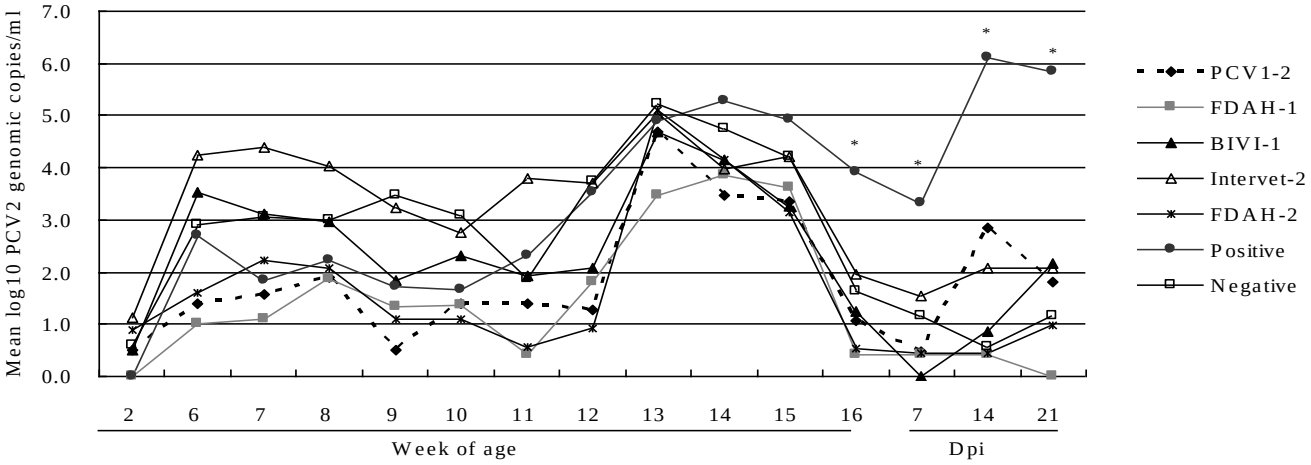
Pre-challenge (2-16 weeks of age). Sixty-five of the 73 pigs were anti-PCV2 IgG positive at 2 weeks of age, with ELISA S/P ratios between 0.48 and 0.67. At 6 weeks of age, the mean group S/P ratios decreased and then increased until 16 weeks of age in all groups. Among vaccinated groups, Intervet-2 and FDAH-2 had the highest and lowest ELISA S/P ratios respectively in the vaccine groups from 6 to 16 weeks of age. There were no significant differences in the mean group PCV2 ELISA S/P ratios between one-dose and two-dose vaccination regimens.

Post challenge (17-19 weeks of age). At 21 days post inoculation (dpi), the PCV2 antibody level increased in all the groups except PCV1-2. The mean group ELISA S/P ratios ranged from 0.76-1.03 in the different vaccine groups, and were 0.70±0.03 for the negative control group and 0.85±0.08 for the positive control group. There were no significant differences in the mean PCV2 ELISA S/P ratios among all the groups, and between one-dose (0.87±0.08) and two-dose (0.89±0.06) vaccination regimens.

3.3. PCV2 DNA in serum

Pre-challenge (2-16 weeks of age). When the piglets arrived at 2 weeks of age, 7/73 (9.6%) pigs had low levels of PCV2 viremia, with group mean $\log_{10}$ PCV2 genomic copy numbers ranging from 0 to 1.1 copies/ml (Fig. 1). From 6 to 16 weeks of age, the incidences of PCV2 viremia ranged from 9.1-100.0% in vaccinated groups, 27.3-100.0% in the non-vaccinated positive control group, and 33.3-100.0% in the negative control group. During 6-16 weeks of age, the group mean $\log_{10}$ PCV2 DNA copy numbers ranged from 0.4 to 4.7 in different vaccinated groups (Fig. 1), and were 0.8-4.1 for one-dose groups and 1.3-5.1 for two-dose groups. All vaccinated groups had significantly ($p<0.05$) lower prevalence of PCV2 viremia and mean $\log_{10}$ PCV2 loads at 16 weeks compared to the positive control group (Fig. 1), with reduction of PCV2 viremia by 49.9-89.5%, which were 78.9% for one-dose vaccines and 68.1% for two-dose vaccines.

Fig 1. Mean group $\log_{10}$ PCV2 DNA loads in different treatment groups during growing period (2-16 weeks of age) and after challenge (7-21 dpi). *Indicates significantly ($p<0.05$) higher PCV2 DNA copy numbers in positive control group.



Post challenge (17-19 weeks of age). After 16 weeks of age, PCV2 DNA loads in the four vaccine groups

remained at low levels (0-2.8 $\log_{10}$ PCV2 copies), whereas mean $\log_{10}$ group PCV2 DNA levels in positive control group increased from 3.3±0.5 (at 7 dpi) to 6.1±0.5 (at 14 dpi) and 5.8±0.6 (at 21 dpi) (Fig. 1). Mean group $\log_{10}$ PCV2 DNA loads in the vaccinated groups were significantly lower ($P<0.01$) than that of the positive control group at 7, 14 and 21 dpi (Fig. 1). The prevalence of PCV2 viremia in positive control group (63.6-100.0%) was significantly higher ($P<0.05$) than that in vaccine groups (0-54.5%). The one-dose and two-dose vaccine groups had comparable PCV2 DNA loads and incidence in the duration of the study with no significant differences. The reduction of PCV2 viremia compared to the positive control group ranged from 53.3% to 100.0% in different vaccine groups. There were no significant differences in the percentage of PCV2 viremia reduction between one-dose (81.4-93.6%) and two-dose (69.8-79.3) vaccination.

3.4. PCV1-2 viremia

PCV1-2 viremia was not detected in the PCV1-2 group at 6 weeks of age (3 weeks after immunization) and thereafter, as well as in other groups at any time-points of the study.

3.5. PRRSV and PPV antibody levels and viremia

All pigs remained negative for PRRSV and PPV antibody until challenge and non-inoculated controls remained negative throughout the length of the study (data not shown). PRRSV antibody was detected in 81.8% (9/11 in the PCV1-2 group) to 100% (10/10 in the Intervet-2 group) of the pigs in vaccine groups, with S/P values ranging from 1.27-1.74. Within the positive control group 81.8% (9/11) where PRRSV seropositive with a mean S/P value of 1.78±0.28. All the challenged pigs were positive for PPV antibodies at 21 dpi, with mean titers of 7,782-10,854 in vaccinated groups and 9,495 in the positive control group. There were no significant differences in PRRSV and PPV antibody levels among different groups or one-dose and two-dose vaccination.

All the pigs except that in negative control group were viremic for PRRSV and PPV at 7dpi. At 14-21 dpi, 100% pigs were viremic for PRRSV and 45-100% were viremic for PPV in negative control group, whereas the viremia percentage in vaccine groups was 90-100% for PRRSV and 64-100% for PPV. The mean $\log_{10}$ PRRSV RNA copies ranged from 4.1 to 7.8 in vaccine groups and 4.9 to 7.5 in positive control group. The mean $\log_{10}$ PPV DNA loads were 2.4-5.7 in vaccine groups and 1.8-5.2 in positive control group. There were no significant differences ($p>0.05$) in PRRSV and PPV prevalence and mean group $\log_{10}$ viral loads when compared among different groups or one-dose and two-dose vaccination.

3.6. Gross and microscopic lesions

No gross or microscopic lesions were observed in the negative control group. At necropsy, mild to moderate gross lesions were found in challenged pigs from both vaccinated and positive control groups, including enlarged lymph nodes and varying degrees of red-to-purple consolidation in lung tissues. The lung gross lesion scores in the vaccinated groups ranged from 1.7% to 5.8% of the lung surface affected by lesions, with no significant differences compared to the negative control (0.9±0.4%) and positive control (2.5±1.3%) groups. Also, no significant differences were observed between one-dose (3.9±1.0%) and two-dose (2.7±1.3%) vaccine administration.

Microscopic lesions, characterized by mild interstitial pneumonia and mild lymphoid depletion and histocytic replacement in lymphoid tissues, were present in all challenged groups. There were no significant differences in mean group scores for any of the evaluated lesions among challenged groups. PCV2 antigen was detected in lymphoid tissues of 3/11 pigs in the positive control group, 0/9 in the negative control group, and 1/53 in the vaccinated groups. The PCV2 IHC scores in vaccinated groups, ranged from 0 to 0.1 and were significantly ($p<0.05$) lower compared to the mean group score of the positive control group (0.5±0.3). There were no significant differences in scores for microscopic lesion and presence and amount of PCV2 antigen in tissues between one-dose and two-dose vaccination regimens.

Discussion

DNA vaccines [37, 38], subunit vaccines [39], recombinant virus vaccines [40, 41], chimeric PCV1-2 vaccines [29, 42] and tissue homogenate vaccines [43] have been described for control of PCV2 infection. Today, PCV2 Cap proteins expressed in baculovirus and an attenuated PCV1-2 chimera are widely used for protection against PCVAD and are commercially available in the U.S. [7]. Although the efficacy of commercial PCV2 vaccines has been evaluated in PCV2 naïve pigs [21, 44] and PCV2 negative pigs [20, 22] under

experimental conditions, the effect of PCV2 viremia on PCV2 vaccine efficacy has to the author's knowledge not been evaluated. This is contrast to the field situation where a high percentage of piglets are born with PCV2 viremia (unpublished data). In this study, we showed for the first time that PCV2 commercial vaccines and a live PCV1-2 vaccine were effective in controlling PCV2 in an experimental PCV2-PRRSV-PPV coinfection model in conventional PCV2 viremic pigs.

Previously, significantly higher levels of IgG and IgM antibodies were detected in vaccinated pigs compared to non-vaccinated pigs with passively-acquired antibodies [22]. In another study also using pigs with passively-acquired antibodies, significantly higher antibody titers (measured by immunoperoxidase monolayer assay, IPMA) were detected in vaccinated pigs compared to non- vaccinated pigs after the second vaccination with a PCV2 subunit vaccine containing the capsid protein [20]. In the current study, the PCV2 antibody levels increased between 6 and 16 weeks of age in all groups. However, no significant differences were observed between vaccinated and non-vaccinated groups. The similar antibody response in vaccinated and non-vaccinated pigs was likely due to active seroconversion against the PCV2 strain present in the piglets. This highlights that interpretation of successful vaccination based on serology is difficult under field conditions.

PRRSV and PPV are two important porcine pathogens capable of enhancing the pathogenicity of PCV2 [8-12], and are often present on PCVAD affected farms [13-16]. In the current study, we evaluated the efficacy of vaccination in the PCV2-PRRSV-PPV coinfection model. If present, the observed PCV2-associated lesions (lymphoid depletion and histiocytic replacement in lymphoid tissues) were mild in all vaccinated and non-vaccinated pigs and not different from negative control pigs. This result is in contrast with that observed in a challenge model using PRRSV-PCV2-SIV [44] or PRRSV and PCV2 coinfection [21]; in these studies PCV2 vaccination effectively reduced PCV2-associated microscopic lesions as compared to non-vaccinated control pigs and likely due to the chronic subclinical PCV2 infection in the majority of the pigs. PCV2 associated lesions usually resolve between 28 and 42 days. Nevertheless, the decreased amount of PCV2 antigen in lymphoid tissues and PCV2 viremia in vaccinated pigs suggested a protective immunity was induced by PCV2 vaccines including situations where PRRSV and PPV coinfections existed.

The live PCV1-2 chimeric vaccine used in the present study was developed by replacing the capsid gene of the PCV1 with that of PCV2a. It has been shown that the PCV1-2 chimera virus was attenuated in pigs but induced protective immunity against PCV2 [29, 46], and it has been demonstrated to be genetically stable both *in vitro* and *in vivo* [47]. In the present study, we further demonstrated that the live PCV1-2 chimera virus used at a dose of 1 ml ($10^{4.0}TCID_{50}$/ml) intramuscularly is efficient in inducing protective immunity against the natural PCV2 infection during the growing period, and in the face of PRRSV and PCV coinfections after challenge, and that this efficiency is comparable to other commercial PCV2 vaccines.

Reduced PCV2 viremia was observed in PCV2 vaccine studies in PCV2 negative pigs under experimental conditions [20-22]. In the present study, our data revealed that all the vaccine regimens were able to keep the animals at a low level of PCV2 viremia, in both naturally PCV2 infected pigs and PCV2-PRRSV-PPV coinfected pigs. The vaccine efficacy in this respect is important because evidence has shown that high levels of PCV2 viremia is related to the development of PCVAD [48, 49].

In summary, in conventional PCV2 positive pigs, all commercially available PCV2 vaccines and the PCV1-2 chimeric live vaccine used were effective in reducing PCV2 viremia during the growing period and after PCV2 challenge. The protective immunity lasted to at least 16 weeks (13 weeks after the first immunization).

References

[1] Tischer I, Gelderblom H, Vettermann W, Koch MA. A very small porcine virus with circular single-stranded DNA. Nature 1982;295(5844):64-6.

[2] Tischer I, Rasch R, Tochtermann G. Characterization of papovavirus-and picornavirus-like particles in permanent pig kidney cell lines. Zentralbl Bakteriol [Orig A] 1974;226(2):153-67.

[3] Tischer I, Mields W, Wolff D, Vagt M, Griem W. Studies on epidemiology and pathogenicity of porcine circovirus. Arch Virol 1986;91(3-4):271-6.

[4] Meehan BM, McNeilly F, Todd D, Kennedy S, Jewhurst VA, Ellis JA, et al. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. J Gen Virol 1998;79 (Pt 9):2171-9.
[5] Clark EG. Post-weaning multisystemic wasting syndrome. Proc Am Assoc Swine Pract 1997;28:499-501.
[6] Allan GM, Ellis JA. Porcine circoviruses: a review. J Vet Diagn Invest 2000;12(1):3-14.
[7] Opriessnig T, Meng XJ, Halbur PG. Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest 2007;19(6):591-615.
[8] Krakowka S, Ellis JA, Meehan B, Kennedy S, McNeilly F, Allan G. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol 2000;37(3):254-63.
[9] Kennedy S, Moffett D, McNeilly F, Meehan B, Ellis J, Krakowka S, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. J Comp Pathol 2000;122(1):9-24.
[10] Allan GM, Kennedy S, McNeilly F, Foster JC, Ellis JA, Krakowka SJ, et al. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. J Comp Pathol 1999;121(1):1-11.
[11] Allan GM, McNeilly F, Ellis J, Krakowka S, Meehan B, McNair I, et al. Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. Arch Virol 2000;145(11):2421-9.
[12] Rovira A, Balasch M, Segales J, Garcia L, Plana-Duran J, Rosell C, et al. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. J Virol 2002;76(7):3232-9.
[13] Pallares FJ, Halbur PG, Opriessnig T, Sorden SD, Villar D, Janke BH, et al. Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). J Vet Diagn Invest 2002;14(6):515-9.
[14] Quintana J, Segales J, Rosell C, Calsamiglia M, Rodriguez-Arrioja GM, Chianini F, et al. Clinical and pathological observations on pigs with postweaning multisystemic wasting syndrome. Vet Rec 2001;149(12):357-61.
[15] Segales J, Calsamiglia M, Rosell C, Soler M, Maldonado J, Martin M, et al. Porcine reproductive and respiratory syndrome virus (PRRSV) infection status in pigs naturally affected with post-weaning multisystemic wasting syndrome (PMWS) in Spain. Vet Microbiol 2002;85(1):23-30.
[16] Rosell C, Segales J, Ramos-Vara JA, Folch JM, Rodriguez-Arrioja GM, Duran CO, et al. Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. The Veterinary record 2000;146(2):40-3.
[17] Rose N, Larour G, Le Diguerher G, Eveno E, Jolly JP, Blanchard P, et al. Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. Prev Vet Med 2003;61(3):209-25.
[18] Pogranichniy RM, Yoon KJ, Harms PA, Sorden SD, Daniels M. Case-control study on the association of porcine circovirus type 2 and other swine viral pathogens with postweaning multisystemic wasting syndrome. J Vet Diagn Invest 2002;14(6):449-56.
[19] Ellis J, Krakowka S, Lairmore M, Haines D, Bratanich A, Clark E, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. J Vet Diagn Invest 1999;11(1):3-14.
[20] Fort M, Sibila M, Allepuz A, Mateu E, Roerink F, Segales J. Porcine circovirus type 2 (PCV2) vaccination of conventional pigs prevents viremia against PCV2 isolates of different genotypes and geographic origins. Vaccine 2008;26(8):1063-71.
[21] Opriessnig T, Madson DM, Prickett JR, Kuhar D, Lunney JK, Elsener J, et al. Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. Veterinary microbiology 2008;131(1-2):103-14.

[22] Opriessnig T, Patterson AR, Elsener J, Meng XJ, Halbur PG. Influence of maternal antibodies on efficacy of porcine circovirus type 2 (PCV2) vaccination to protect pigs from experimental infection with PCV2. Clin Vaccine Immunol 2008;15(3):397-401.
[23] Fachinger V, Bischoff R, Jedidia SB, Saalmuller A, Elbers K. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Vaccine 2008;26(11):1488-99.
[24] Cline G, Wilt V, Diaz E, Edler R. Efficacy of immunising pigs against porcine circovirus type 2 at three or six weeks of age. The Veterinary record 2008;163(25):737-40.
[25] Segales J, Urniza A, Alegre A, Bru T, Crisci E, Nofrarias M, et al. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) improves clinical, pathological and virological outcomes in postweaning multisystemic wasting syndrome affected farms. Vaccine 2009.
[26] Horlen KP, Dritz SS, Nietfeld JC, Henry SC, Hesse RA, Oberst R, et al. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. J Am Vet Med Assoc 2008;232(6):906-12.
[27] Kixmoller M, Ritzmann M, Eddicks M, Saalmuller A, Elbers K, Fachinger V. Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. Vaccine 2008;26(27-28):3443-51.
[28] Sorden SD, Harms PA, Nawagitgul P, Cavanaugh D, Paul PS. Development of a polyclonal-antibody-based immunohistochemical method for the detection of type 2 porcine circovirus in formalin-fixed, paraffin-embedded tissue. J Vet Diagn Invest 1999;11(6):528-30.
[29] Fenaux M, Opriessnig T, Halbur PG, Meng XJ. Immunogenicity and pathogenicity of chimeric infectious DNA clones of pathogenic porcine circovirus type 2 (PCV2) and nonpathogenic PCV1 in weanling pigs. Journal of virology 2003;77(20):11232-43.
[30] Nawagitgul P, Harms PA, Morozov I, Thacker BJ, Sorden SD, Lekcharoensuk C, et al. Modified indirect porcine circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV. Clin Diagn Lab Immunol 2002;9(1):33-40.
[31] Mengeling WL, Ridpath JF, Vorwald AC. Size and antigenic comparisons among the structural proteins of selected autonomous parvoviruses. The Journal of general virology 1988;69 (Pt 4):825-37.
[32] Halbur PG, Paul PS, Frey ML, Landgraf J, Eernisse K, Meng XJ, et al. Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Veterinary pathology 1995;32(6):648-60.
[33] Opriessnig T, Yu S, Gallup JM, Evans RB, Fenaux M, Pallares F, et al. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Veterinary pathology 2003;40(5):521-9.
[34] Opriessnig T, McKeown NE, Harmon KL, Meng XJ, Halbur PG. Porcine circovirus type 2 infection decreases the efficacy of a modified live porcine reproductive and respiratory syndrome virus vaccine. Clin Vaccine Immunol 2006;13(8):923-9.
[35] Opriessnig T, Fenaux M, Thomas P, Hoogland MJ, Rothschild MF, Meng XJ, et al. Evidence of breed-dependent differences in susceptibility to porcine circovirus type-2-associated disease and lesions. Veterinary pathology 2006;43(3):281-93.
[36] Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ, Halbur PG. Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and porcine circovirus type 2. Veterinary pathology 2004;41(6):624-40.
[37] Shen HG, Zhou JY, Huang ZY, Guo JQ, Xing G, He JL, et al. Protective immunity against porcine circovirus 2 by vaccination with ORF2-based DNA and subunit vaccines in mice. J Gen Virol 2008;89(Pt 8):1857-65.
[38] Kamstrup S, Barfoed AM, Frimann TH, Ladekjaer-Mikkelsen AS, Botner A. Immunisation against PCV2 structural protein by DNA vaccination of mice. Vaccine 2004;22(11-12):1358-61.
[39] Blanchard P, Mahe D, Cariolet R, Keranflec'h A, Baudouard MA, Cordioli P, et al. Protection of swine against post-weaning multisystemic wasting syndrome (PMWS) by porcine circovirus type 2 (PCV2) proteins. Vaccine 2003;21(31):4565-75.

[40] Song Y, Jin M, Zhang S, Xu X, Xiao S, Cao S, et al. Generation and immunogenicity of a recombinant pseudorabies virus expressing cap protein of porcine circovirus type 2. Vet Microbiol 2007;119(2-4):97-104.
[41] Wang X, Jiang P, Li Y, Jiang W, Dong X. Protection of pigs against post-weaning multisystemic wasting syndrome by a recombinant adenovirus expressing the capsid protein of porcine circovirus type 2. Vet Microbiol 2007;121(3-4):215-24.
[42] Fenaux M, Opriessnig T, Halbur PG, Elvinger F, Meng XJ. Two amino acid mutations in the capsid protein of type 2 porcine circovirus (PCV2) enhanced PCV2 replication in vitro and attenuated the virus in vivo. J Virol 2004;78(24):13440-6.
[43] Baker RB, Madson DM, Patterson AR, Opriessnig T. Evaluation of the safety of four porcine circovirus type 2 tissue homogenate vaccines in a pig bioassay. Vet Rec 2009;165(9):262-5.
[44] Opriessnig T, Patterson AR, Madson DM, Pal N, Halbur PG. Comparison of efficacy of commercial one dose and two dose PCV2 vaccines using a mixed PRRSV-PCV2-SIV clinical infection model 2-3-months post vaccination. Vaccine 2009;27(7):1002-7.
[45] Sorden SD. Update on porcine circovirus and postweaning multisystemic wasting syndrome (PMWS). Swine Health Prod 2000;8:133-6.
[46] Fenaux M, Opriessnig T, Halbur PG, Elvinger F, Meng XJ. A chimeric porcine circovirus (PCV) with the immunogenic capsid gene of the pathogenic PCV type 2 (PCV2) cloned into the genomic backbone of the nonpathogenic PCV1 induces protective immunity against PCV2 infection in pigs. J Virol 2004;78(12):6297-303.
[47] Gillespie J, Juhan NM, DiCristina J, Key KF, Ramamoorthy S, Meng XJ. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) is genetically stable in vitro and in vivo. Vaccine 2008;26(33):4231-6.
[48] Larochelle R, Magar R, D'Allaire S. Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. Can J Vet Res 2003;67(2):114-20.
[49] Meerts P, Misinzo G, Lefebvre D, Nielsen J, Botner A, Kristensen CS, et al. Correlation between the presence of neutralizing antibodies against porcine circovirus 2 (PCV2) and protection against replication of the virus and development of PCV2-associated disease. BMC Vet Res 2006;2:6.