

SWINE HEALTH

Title: Comparison of the efficacy of sow vaccination versus piglet vaccination for PCV2 and evaluation of the impact of revaccination with a homologous PCV2 vaccine – NPB #07-217

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Industry Summary:

The objectives of this study were (1) to compare the efficacy of two different PCV2 vaccination protocols (maternally derived antibodies versus piglet vaccination) in a conventional PCV2 growing pig challenge model and (2) to evaluate the efficacy of concurrent dam and piglet homologous PCV2 vaccination. Two different commercially available vaccines (VAC1; VAC2) were used side by side. Seventy-eight piglets born to vaccinated or non-vaccinated sows were divided into 8 groups. A proportion of the pigs with and a proportion of the pigs without passively acquired antibodies were vaccinated at 21 days of age. All pigs except negative controls were challenged with PCV2b at 35 days post-vaccination and necropsied at 21 days post-challenge (dpc). The data indicate that both vaccine regimens had similar efficacies in reducing PCV2 viral loads and antigen levels in the growing pigs. Interestingly, dam vaccination alone did result in significantly ($P < 0.05$) lower anti-PCV2-antibodies levels at challenge in piglets from dams immunized with VAC2 compared to piglets derived from VAC1 immunized dams. When data obtained from the growing piglets that were vaccinated with VAC1 or VAC2 were compared, antibody levels and reduction of incidence of PCV2 antigen were not different; however, piglets vaccinated with VAC2 had reduced PCV2 DNA genomic copies in serum by 21 dpc. Homologous revaccination of piglets derived from vaccinated dams did not appear to affect vaccine efficacy as piglets in these groups had anti-PCV2-antibody levels and PCV2 genomic copies similar to the groups where vaccine was administered to the piglets only.

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Scientific Abstract (limit to one page)

The objectives of this study were (1) to compare the efficacy of two different PCV2 vaccination protocols (maternally derived antibodies versus piglet vaccination) in a conventional PCV2 growing pig challenge model and (2) to evaluate the efficacy of concurrent dam and piglet homologous PCV2 vaccination. Two different commercially available vaccines (VAC1; VAC2) were used side by side. Seventy-eight piglets born to vaccinated or non-vaccinated sows were divided into 8 groups. A proportion of the pigs with and a proportion of the pigs without passively acquired antibodies were vaccinated at 21 days of age. All pigs except negative controls were challenged with PCV2b at 35 days post-vaccination and necropsied at 21 days post-challenge (dpc). The data indicate that both vaccine regimens had similar efficacies in reducing PCV2 viral loads and antigen levels in the growing pigs. Interestingly, dam vaccination alone did result in significantly ($P < 0.05$) lower anti-PCV2-antibodies levels at challenge in piglets from dams immunized with VAC2 compared to piglets derived from VAC1 immunized dams. When data obtained from the growing piglets that were vaccinated with VAC1 or VAC2 were compared, antibody levels and reduction of incidence of PCV2 antigen were not different; however, piglets vaccinated with VAC2 had reduced PCV2 DNA genomic copies in serum by 21 dpc. Homologous revaccination of piglets derived from vaccinated dams did not appear to affect vaccine efficacy as piglets in these groups had anti-PCV2-antibody levels and PCV2 genomic copies similar to the groups where vaccine was administered to the piglets only.

Introduction

Porcine circovirus type 2 (PCV2) has been associated with many clinical signs in growing pigs including lymphadenopathy, wasting, pneumonia, and diarrhea (Allan et al., 2000a). Recently, PCV2-associated disease (PCVAD) has rapidly spread across North America (Horlen et al., 2007; Cheung et al., 2007). The disease now affects most swine production regions worldwide, causing devastating production and economic losses within the industry. In response to the rapid spread of PCVAD, commercial vaccines for PCV2 became available in North America in 2006. At this time, three different conditionally or fully licensed commercial PCV2 vaccines for growing pigs are on the market in the United States. In addition, a vaccine approved for use in sows became available in Canada (Opriessnig et al., 2007).

While vaccination of sows better ensures that piglets receive high amounts of maternal antibodies, previous studies have shown that passively-acquired antibodies alone may not provide complete protection to piglets (McKeown et al., 2005; Opriessnig et al., 2008a). In a previous study, half of the pigs with maternal antibodies of various levels were vaccinated against PCV2 and the remaining pigs remained non-vaccinated (Opriessnig et al., 2008a). The non-vaccinated pigs developed PCV2-viremia regardless of their antibody status, whereas the vaccinated pigs had significantly reduced incidence and length of PCV2 viremia (Opriessnig et al., 2008a). Similar results were obtained using a different PCV2 vaccine product in pigs with passively acquired antibodies (Fort et al., 2008).

Due to issues such as vaccine availability and cost per dose, some practitioners have started to vaccinate sow herds against PCV2 in an off-label fashion and are decreasing the use of the vaccine in growing pigs. It is expected that various combinations of regimens for sow and piglet vaccination will continue in the future. Both dams and their offspring will likely be vaccinated with the same vaccine to prevent or diminish PCVAD. There is concern from practitioners that vaccine-derived maternal antibodies could interfere with the active piglet vaccination, making it less effective, especially if the same vaccine is used to vaccinate both the dams and their offspring.

Objectives

- To compare the efficacy of sow PCV2 vaccination versus piglet PCV2 vaccination in a conventional PCV2 pig challenge model
- To evaluate the impact and efficacy of vaccinating sows and piglets with a homologous PCV2 vaccine
- To further evaluate the effect of PCV2b challenge on vaccine efficacy of PCV2a-based products

Materials and Methods

Animals, housing, and experimental design

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee. Nine crossbred, specific-pathogen-free (SPF), conventional sows free of antibodies to PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), and porcine parvovirus (PPV) were used in this study. The dams were divided into three groups: At 28 and at 93 days of gestation, two dams were vaccinated with 2ml of VAC1 (Merial; Lot number L207864), three dams were vaccinated with 1ml of VAC2 (BIVI; Serial number 309-024), and four dams remained non-vaccinated. The litter sizes at birth were 18 (12 liveborns, 3 stillborns, 3 mummies) and 10 (10 liveborns) for dams vaccinated with VAC1 and 12 (11 liveborns and 1 stillborn), 18 (14 liveborns, 2 stillborns, 2 mummies), and 13 (11 liveborns, 2 stillborns) for dams vaccinated with VAC2. The piglets were allowed to suckle colostrum from their respective dams with cross-fostering within groups to even out litter sizes. At two weeks of age all surviving piglets (VAC1=16/22 and VAC2=22/36) were weaned from these sows. In addition, 40 piglets were weaned from the non-vaccinated sows.

The 78 pigs were blocked by dam vaccination status, randomly divided into 8 groups (piglet vaccination: VAC1-P and VAC2-P; dam vaccination: VAC1-D and VAC2-D; or both dam and piglet vaccination: VAC1-D/P and VAC2-D/P), and transported to the Livestock Infectious Disease Isolation Facility at Iowa State University, Ames, Iowa. All challenged pigs were randomly assigned to one of two rooms each containing six 2.5 × 3.6 m raised wire decks equipped with one nipple drinker and one self-feeder. The ten non-challenged negative control pigs were housed in a similar separate room distributed into two decks.

At approximately 21 days of age, piglets in VAC1-P, VAC1-D/P, VAC2-P, and VAC2-D/P were vaccinated with one of two commercial PCV2 vaccines. At 56 days of age, all pigs except negative controls were challenged with a PCV2b isolate. The pigs were monitored daily for clinical signs of disease, and weighed and bled weekly. Twenty-one days post-PCV2 challenge (dpc 21), all pigs were necropsied and the tissues were evaluated for macroscopic and microscopic lesions.

Vaccination

Two different commercially available PCV2 vaccines were used in this study: VAC1 (CIRCOVAC®; Merial, Lyon, France, Lot number L207864) contains killed PCV2a and is licensed for achieving high levels of passively acquired immunity in growing pigs by vaccinating breeding animals. VAC1 is administered twice as 2ml intramuscular injections at least 3-4 weeks apart for primary vaccination. As recommended by the manufacturer, the reconstituted vaccine was used within 3 hours. VAC2 (Ingelvac® CircoFLEX™; Boehringer Ingelheim Vetmedica, Inc.; serial number 309-024) contains PCV2a antigen expressed in a Baculovirus vector and is licensed for usage in pigs 3 weeks of age and older administered as one dose (1ml) intramuscular injection. Dams and piglets were vaccinated intramuscularly into the right neck. At 28 and again at 93 days of gestation, the dams used to obtain VAC1-D and VAC1-D/P piglets received 2 ml of CIRCOVAC®, and the dams used to obtain VAC2-D and VAC2-D/P piglets received 1 ml of Ingelvac® CircoFLEX™. At 21 days of age, VAC1-P and VAC1-D/P piglets received 2 ml of CIRCOVAC®, and VAC2-P and VAC2-D/P piglets received 1 ml of Ingelvac® CircoFLEX™.

Challenge

Piglet PCV2 challenge was conducted at 56 days of ages (35 days after vaccination of the growing pigs). PCV2b isolate NC-16845 (Opriessnig et al., 2008b) at a dose of $10^{4.2}$ TCID₅₀ was used for the challenge. Each pig, except for the negative controls, received 2ml of the inoculum intramuscularly into the right neck and 3 ml intranasally by slowly dripping the inoculum into both nostrils. Successful challenge using this model was demonstrated by seroconversion and viremia in the positive controls.

Serology

Blood samples were collected in the first week after the piglets were born and weekly thereafter until necropsy. The serum was tested by an in-house ORF2-based PCV2 IgG ELISA (Nawagitgul et al., 2002).

Samples were considered positive if the calculated S/P ratio was 0.2 or greater (Nawagitgul et al., 2002). A fluorescence focus neutralization assay was done on the day of challenge in order to determine the presence of neutralizing antibodies (NA) against PCV2 according to the standard Iowa State University (ISU) Veterinary Diagnostic Laboratory operating protocol (Pogranichniy et al., 2000). PCV2 isolate ISU-98-15237 was used in this assay. A neutralizing antibody assay titer was considered positive if higher than the average titers obtained in negative control animals.

Clinical evaluation

Following PCV2-inoculation, the pigs were monitored daily for clinical signs including sneezing, lethargy, coughing, and all pigs were weighed once a week.

PCV2 DNA quantification

DNA was extracted from serum samples collected on the day of challenge and on days 7, 14, and 21 dpc using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA). DNA-extracts were used for quantification of PCV2 genomic DNA copy numbers by real-time PCR as described previously (Opriessnig et al., 2003).

Necropsy

All pigs were euthanized by intravenous phenobarbital overdose and necropsied on 21 dpc. The total amount of macroscopic lung lesions (ranging from 0 to 100%) was estimated and scored (Halbur et al., 1995). Additionally, the size of lymph nodes ranging from 0 (normal) to 3 (four times the normal size) were estimated and recorded (Opriessnig et al., 2006). Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, mesenteric, and external iliac), tonsil, thymus, ileum, kidney, colon, spleen, and liver were collected at necropsy and fixed in 10% neutral-buffered formalin and routinely processed for histological examination.

Histopathology

Microscopic lesions were evaluated by a pathologist blinded to the group designation of animal tissues. 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, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 (none) to 3 (severe). Lymphoid tissues including lymph nodes (tracheobronchiolar, mesenteric, mediastinal, superficial inguinal, and external iliac), tonsil, and spleen were evaluated for the presence of lymphoid depletion ranging from 0 (normal) to 3 (severe) and histiocytic inflammation and replacement of follicles ranging from 0 (normal) to 3 (severe) (Opriessnig et al., 2004). The overall microscopic lymphoid lesions score which accounts for 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).

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, external iliac, and mesenteric), tonsil, spleen, and thymus using a rabbit polyclonal antiserum (Sorden et al., 1999). PCV2-antigen scoring was done by a pathologist blinded to animal group designation. Scores ranged from 0 (no signal) to 3 (more than 50% of the lymphoid follicles contain cells with PCV2-antigen staining) (Opriessnig et al., 2004).

Statistical analysis

The general linear model (glm) procedure of SAS (SAS Institute, Inc., Cary, North Carolina, USA) was used to assess the effect of time on the following repeated measures data: \log_{10} genomic copies/ml, anti-PCV2-IgG antibody levels and neutralizing antibody levels. A multivariate analysis of variance (ANOVA) was performed using group as the factor and time as the repeated variable in the model. If a significant effect of time was identified using this model, the glm procedure of SAS with the Bonferroni (Dunn) t test was performed

using group as the dependent fixed factor in the model to identify pair-wise differences between group means at each time point. For non-repeated measures of data including average daily gain and histopathology lesion scores, the glm procedure of SAS with the Bonferroni (Dunn) t test was performed using group as the dependent fixed factor in the model to identify pair-wise differences between group means.

Results

Clinical presentation

Clinical disease was not observed in any of the pigs for the duration of the study. The average weight in lbs (\pm standard error) for each group at the time of vaccination (21 days of age) was as follows: Negative controls, 58.9 ± 3.6 ; positive controls, 56.8 ± 3.4 ; VAC1-P, 55.5 ± 3.3 ; VAC1-D/P, 45.6 ± 3.6 ; VAC1-D, 45.1 ± 3.6 ; VAC2-P, 55.7 ± 1.9 ; VAC2-D/P, 51.8 ± 1.9 ; and VAC2-D, 56.0 ± 2.2 . VAC1-D had a significantly ($P < 0.05$) lower mean body weight compared to the negative controls. No other differences were noted among treatment groups.

The average weight in lbs (\pm standard error) for each group at the time of challenge (56 days of age) was as follows: Negative controls, 68.3 ± 3.9 ; positive controls, 65.7 ± 3.8 ; VAC1-P, 67.1 ± 3.1 ; VAC1-D/P, 55.1 ± 4.4 ; VAC1-D, 56.2 ± 4.3 ; VAC2-P, 65.6 ± 2.0 ; VAC2-D/P, 60.8 ± 2.7 ; and VAC2-D, 66.5 ± 2.3 . There were no significant differences among groups.

The average daily weight gain in lbs (\pm standard error) for each group from challenge to necropsy was as follows: Negative controls, 1.68 ± 0.1 ; positive controls, 1.40 ± 0.2 ; VAC1-P, 1.40 ± 0.4 ; VAC1-D/P, 1.47 ± 0.1 ; VAC1-D, 1.69 ± 0.1 ; VAC2-P, 1.63 ± 0.1 ; VAC2-D/P, 1.42 ± 0.2 ; and VAC2-D, 1.66 ± 0.1 . No significant differences were noted among the groups.

Macroscopic lesions

No remarkable gross lesions were observed. Individual vaccinated and non-vaccinated pigs had slightly enlarged mediastinal lymph nodes (data not shown).

Anti-PCV2-IgG antibody levels

Non-vaccinated dams remained negative, and as expected the vaccinated sows seroconverted to PCV2 (data not shown). At the time of farrowing, the mean colostrum S/P ratio of dams vaccinated with VAC1 was 0.56 ± 0.02 . Dams vaccinated with VAC2 had a mean colostrum S/P ratio of 0.43 ± 0.05 and the non-vaccinated dams had a mean colostrum S/P ratio of 0.01 ± 0.00 . The negative control piglets remained negative for PCV2 antibodies until termination of the study (data not shown) whereas the positive control piglets seroconverted to PCV2 by 14 dpc, indicating successful challenge.

When the piglets were 21 days of age (day of active immunization), all piglets from negative dams ($n=40$) were seronegative (mean S/P ratio 0.00 ± 0.01) and all piglets from vaccinated sows ($n=38$) had passively derived PCV2-antibodies. The average anti-PCV2 IgG S/P ratio for piglets derived from dams vaccinated with VAC1 was 0.93 ± 0.02 and the average S/P ratio for piglets derived from dams vaccinated with VAC2 was 0.89 ± 0.02 . At PCV2 challenge (56 days of age) the average PCV2 IgG S/P ratio for piglets derived from dams vaccinated with VAC1 was significantly ($P < 0.05$) higher than the average S/P ratio for piglets derived from dams vaccinated with VAC2. For VAC1, after challenge, no significant differences in anti-PCV2-IgG serum levels were detected in piglets regardless of whether the sow, the piglet or both the sow and the piglet were vaccinated. In contrast for VAC2, vaccination of both the dam and the piglet resulted in a significantly greater serological response in piglets than vaccination of the sow alone.

Neutralizing antibodies

The log transformed group mean NA titers \pm standard error at the day of challenge were as follows: Negative controls, 2.2 ± 0.2 ; positive controls, 2.2 ± 0.1 ; VAC1-P, 2.8 ± 0.2 ; VAC1-D/P 3.1 ± 0.1 ; VAC1-D, 3.1 ± 0.1 ; VAC2-P, 2.5 ± 0.1 ; VAC2-D/P 2.5 ± 0.1 ; and VAC2-D 2.3 ± 0.1 . Vaccination of the dams with VAC1 resulted in significantly ($P < 0.05$) higher mean NA titers in the offspring in comparison to vaccination of the

dam alone or both the dam and piglet using VAC2. However, there was no difference between vaccinating the dam, the piglet or both the dam and the piglet using either VAC1 or VAC2.

Incidence and amount of PCV2 DNA in serum

PCV2 DNA was not detected in any pig at the day of challenge (0 dpc) or in pigs in the negative control group at any time point (data not shown). Data indicates that at 7 dpc, vaccination of dams with VAC1 significantly ($P < 0.05$) reduced viremia in the offspring in comparison to VAC1 piglet vaccination alone or the combination of VAC1 piglet and VAC1 sow vaccination. In contrast, when the VAC2 product was used, piglet vaccination or the combination of sow and piglet vaccination significantly ($P < 0.05$) reduced viremia in comparison to sow vaccination alone at dpc 14, and 21 (FIG 5). The incidence of PCV2 viremia by 21 dpc was reduced by 20% in the VAC1-P group (8/10), 57.1% in VAC1-D/P (3/7), 44.4% in VAC1-D (5/9), 60% in VAC2-P (4/10), 50% in VAC2-D/P (6/12), and 0% in VAC2-D (10/10) compared to the positive controls (10/10).

Comparing data from VAC1-D and VAC2-D, vaccination of the dams with VAC1 resulted in significantly ($P < 0.05$) lower amounts of PCV2 DNA in sera of their offspring at 7, 14, and 21 dpc. However, when the two vaccines were compared in the growing pigs (VAC1-P, VAC2-P), VAC2-P had significantly ($P < 0.05$) lower amounts of PCV2 DNA in sera at 21 dpc. In contrast, there was no difference between vaccine products when homologous re-vaccination was evaluated (VAC1-D/P versus VAC2-D/P).

Microscopic lesions

Individual pigs in all groups had mild focal interstitial pneumonia (highest score 1 out of 6) without significant differences in incidence among groups (data not shown). The reduction of incidence of PCV2 antigen detection was 100% for VAC1-P, 82% for VAC1-D/P, 86% for VAC1-D, 100% for VAC2-P and VAC2-D/P, and 0% for VAC2-D compared to the positive controls.

Discussion

In response to losses related to PCVAD, commercial vaccines against PCV2 infection became available in North America in 2006 and are now widely used (Fort et al., 2008; Fachinger et al., 2008; Kixmoller et al., 2008; Horlen, 2008). In this study, two commercially available products were used to either immunize pigs, dams, or both. One vaccine (VAC1) is licensed for immunizing breeding animals thereby inducing passive immunity in growing pigs. The other vaccine (VAC2) is licensed for induction of active immunity in growing pigs. Due to these differences, VAC1 was used off label on piglets and VAC2 was used off label on dams. Since there was no recommendation for VAC1 in growing pigs, a single 2ml dose (as recommended for this product in sows) was used (similar to VAC2). In contrast, two 1 ml doses of VAC2 were used (as recommended for VAC1 in growing pigs). The outcomes may have been influenced by the off-label use. Furthermore, the number of vaccinated sows per product (n=2 to 3) was limited by lack of availability of PCV2 naïve dams and an effect of the litter on the outcome cannot completely be ruled out. However, pigs from vaccinated sows had similar amounts of passively acquired antibodies in serum at the time of vaccination indicating that the uptake of colostrum was similar among pigs and groups and did not influence the outcomes of this study.

Both of the PCV2 vaccines used have the same intended outcome: protection of growing pigs to develop PCVAD mainly by reducing clinical signs, macroscopic and microscopic lesions, and the amount of PCV2 present in the pigs. The mechanism of action is induction of active immunity in one of the products (VAC2) versus enhancement of transfer of passive immunity in the other product (VAC1). The main advantage of using a sow vaccination versus a piglet vaccination is the reduction of cost and labor. One sow is vaccinated versus the entire litter. However, as the sow vaccination works in enhancing IgG levels in the colostrum, its efficacy will depend on the piglet having access to sufficient amount of colostrum during the first 1-2 days of life and on the half-life of the passively acquired antibodies. Similarly, the efficacy of the piglet vaccination depends on compliance with procedures to ensure that each individual pig is vaccinated which may be challenging in some production systems. In field studies, VAC2 has been shown to be effective in reducing the mean PCV2 viral load, in reducing the mean duration of PCV2 viremia, and in improving the average daily weight gain while

reducing the time to market when compared to non-vaccinated pigs (Fachinger et al., 2008; Kixmoller et al., 2008).

Dam vaccination alone did result in significantly ($P < 0.05$) lower anti-PCV2-antibody levels at challenge in dams immunized with VAC2 compared to those immunized with VAC. In addition, piglets from the VAC1-D group also had reduced PCV2 viremia in serum and reduced prevalence of PCV2 antigen detected in lymphoid tissues. Since VAC1 is licensed for usage in breeding animals this should be expected; however, in this study, the growing pigs were challenged at 56 days of age and it is unknown if the piglets still would have been protected at a later challenge time.

When VAC1 and VAC2 were administered to growing pigs and compared side by side, antibody levels were significantly ($P < 0.05$) lower in VAC2-P groups by 7 dpc with similar PCV2 DNA levels in both groups. However, by 21 dpc, VAC2-P piglets had significantly ($P < 0.05$) reduced PCV2 DNA genomic copies in serum. This lack of correlation between antibody levels and PCV2 viral load may be a result of induction of a cellular immune response unrelated to the antibody response. It is of interest, that there was no obvious correlation of anti-IgG and neutralizing antibodies; however, only serum obtained at the day of challenge was tested for presence of neutralizing antibodies.

The limitations of this study include the lack of additional co-stimulation such as PRRSV (Allan et al., 2000b; Rovira et al., 2002), PPV (Allan et al., 1999), *Mycoplasma hyopneumoniae* (Opriessnig et al., 2004) or keyhole limpet hemocyanin in incomplete Freund's adjuvant (Krakowa et al., 2001) which all have been shown to enhance PCV2 replication in the experimental pig model. Addition of a co-stimulation could have increased the severity of PCV2-associated lesions and disease. Furthermore, the pigs in this study were challenged at 8 weeks of age whereas exposure in the field is likely quite variable, often later and repeated. Finally, the sows utilized in this study were seronegative for PCV2. Under field conditions, most sows are seropositive at the time of vaccination which may impact successful sow vaccination and/or transfer of naturally acquired immunity to the offspring.

Based on the results of this study, vaccination of both dam and piglet with homologous vaccine did not appear to have an adverse affect post PCV2 challenge. Compared to the positive controls, reduction of PCV2 viremia was significant in VAC1-D/P and VAC2-D/P with a similar effect on reduction of PCV2-antigen load in lymphoid tissues. It is important for practitioners and producers to know that under the conditions of this study, vaccination of growing pigs with the same vaccine used in the dams did not negatively influence vaccine efficacy.

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