RESEARCH REPORT
SWINE HEALTH

Title: The efficacy of sow vaccination with commercial PCV2 vaccines and an experimental live PCV1-2 product – **NPB # 09-177**

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Industry Summary (suitable for immediate public release; non-technical audience)

Porcine circovirus type 2 (PCV2) is associated with reproductive failure in the field and because of this PCV2 vaccines are now being used in some breeding herds. Research has demonstrated that PCV2 is capable of crossing the placental barrier and infecting fetuses resulting in reproductive failure (abortions, mummified fetuses, stillborn and weakborn pigs). The objectives of this study were to determine 1) if there are differences in levels of protection against PCV2 challenge in dams vaccinated with different PCV2 vaccine doses, 2) determine if dam vaccination with either an inactivated or a live chimeric PCV2 vaccine is sufficient to reduce PCV2 viremia and presence of PCV2 antigen in fetal tissues, and 3) determine if there are differences in efficacy between PCV2 vaccines. Thirty-five sows of different parities (parity 1-7) were randomly divided into 6 groups: negative controls (n=5), positive controls (n=6), 1 dose inactivated vaccine and PCV2 challenged (*1d-vaccine:PCV2*; n=6), 2 dose inactivated vaccine and PCV2 challenged, (*2d-vaccine:PCV2*; n=6), 1 dose live vaccine and unchallenged (*1d-live-vaccine*; n=6), and 1 dose live vaccine and PCV2 challenged (*1d-live-vaccine:PCV2*; n=6). A portion of the sows were challenged with PCV2 by using semen spiked with PCV2 (positive controls, *1d-vaccine:PCV2*; *2d-vaccine:PCV2*, and *1d-live-vaccine:PCV2*). Four of 35 sows became pregnant. Serum from both sows and fetuses was tested by quantitative real-time PCR for the presence and quantity of PCV2 and PCV1-2 DNA and for the presence of PCV2-specific antibodies by ELISA. The results indicate that the inactivated PCV2 vaccine is capable of inducing higher levels of PCV2-specific antibodies than the live PCV2 vaccine in sows, but that all vaccination strategies tested provided almost complete protection against PCV2 viremia in sows and piglets and are capable of reducing PCV2 antigen in tissues. In conclusion, vaccination was successful in reducing PCV2 viremia and PCV2 antigen in tissues of piglets indicating that both the inactivated and the live PCV2 vaccines are successful in inducing an antibody response and decreasing PCV2 viremia and evidence suggests that live PCV2 vaccines could potentially be used effectively in breeding herds.

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III. Keywords: Porcine circovirus type 2 (PCV2); breeding herd; vaccination

Scientific Abstract

Porcine circovirus type 2 (PCV2) is associated with reproductive failure in the field and because of this PCV2 vaccines are now being used in some breeding herds. Research has demonstrated that PCV2 is capable of crossing the placental barrier and infecting fetuses which can result in fetal death. The objectives of this study were to determine 1) if there are differences in levels of protection against PCV2 challenge in dams vaccinated with different PCV2 vaccine doses, 2) determine if dam vaccination with either an inactivated or a live chimeric PCV2 vaccine is sufficient to reduce PCV2 viremia and presence of PCV2 antigen in fetal tissues, and 3) determine if there are differences in efficacy between PCV2 vaccines. Thirty-five sows of different parities (parity 1-7) were randomly divided into 6 groups: negative controls (n=5), positive controls (n=6), 1 dose inactive vaccine and PCV2 challenged (*1d-vaccine:PCV2*; n=6), 2 dose inactivated vaccine and PCV2 challenged, (*2d-vaccine:PCV2*; n=6), 1 dose live vaccine and unchallenged (*1d-live-vaccine*; n=6), and 1 dose live vaccine and PCV2 challenged (*1d-live-vaccine:PCV2*; n=6). A portion of the sows were challenged with PCV2 by using semen spiked with PCV2 (positive controls, *1d-vaccine:PCV2*; *2d-vaccine:PCV2*, and *1d-live-vaccine:PCV2*). Four of 35 sows became pregnant. Serum from both sows and fetuses was tested by quantitative real-time PCR for the presence and quantity of PCV2 and PCV1-2 DNA and for the presence of PCV2-specific antibodies by ELISA. The results indicate that the inactivated PCV2 vaccine is capable of inducing higher levels of PCV2-specific antibodies than the live PCV2 vaccine in sows; however, both the commercial killed vaccines and the attenuated live chimeric vaccine provided excellent protection against PCV2 viremia in sows and piglets and are capable of reducing PCV2 antigen in tissues. In conclusion, vaccination was successful in reducing PCV2 viremia and PCV2 antigen in tissues of piglets indicating that both the inactivated and the live PCV2 vaccines are successful in inducing an antibody response and decreasing PCV2 viremia and evidence suggests that live PCV2 vaccines could potentially be used effectively in breeding herds.

Introduction

Porcine circovirus (PCV) is a member of the *Circoviridae* family and the genus Circovirus. It is a non-enveloped, single stranded DNA virus with a circular genome. The genome of PCV2 contains two major open reading frames (ORFs): ORF 1 which encodes for a protein essential for viral replication, and ORF 2 which encodes for the capsid protein (Allan and Ellis, 2000; Gillespie et al., 2009). Two main genotypes of PCV have been identified: PCV type 1 (PCV1) and PCV type 2 (PCV2) which share approximately 83% nucleotide homology in ORF1 but only 67% in ORF2 (Morozov et al., 1998).

PCV1 was first identified as a contaminate of a continuous porcine kidney cell line (PK-15) in 1974 (Tischer et al., 1974; Tischer et al., 1982). Despite being widespread in the swine population, PCV1 has been shown to be non-pathogenic (Allan and Ellis, 2000; Tischer et al., 1986). PCV2 is the causative agent of a group of diseases collectively called porcine circovirus associated disease (PCVAD). PCVAD includes post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), PCV2-associated reproductive failure, and PCV2-associated enteric disease in growing pigs (Gillespie et al., 2009).

Recently, a new PCV genotype was isolated from pigs and designated PCV1-2. This genotype contains the ORF1 of PCV1 and the ORF2 of PCV2a. This virus was isolated from three pigs in Canada from three separate diagnostic cases in 2008 and 2009 (Gagnon et al., 2010). The source of this new genotype is in question. Natural recombination between PCV1 and PCV2 have been suggested as a potential explanation

(Gagnon et al., 2010). Another possible explanation is that the virions may have come from improperly inactivated chimeric PCV2 vaccine widely used in Canada at the time of identification of the PCV1-2a isolates. However, it has been shown that a similar chimeric PCV1-2 strain is non-pathogenic and could potentially be used as a modified live chimeric PCV2 vaccine (Fenaux et al., 2003; Fenaux et al., 2004).

PCV2 can be further divided into several subtypes. The most important subtypes which are both prevalent worldwide include PCV2a which was the predominant strain in the pig population before 2000, and PCV2b which has replaced PCV2a in most herds and is currently the predominant PCV2 subtype in North America (Gillespie et al., 2009). The main difference between PCV2a and PCVb is located in ORF2 and known as the signature motive. In addition, the genome of PCV2a is 1768 nucleotides in length whereas that of PCV2b is 1767 nucleotides in length. Comparative pathogenicity studies comparing isolates of the two subtypes side by side in pigs have failed to show any differences under experimental conditions (Gillespie et al., 2009; Olvera et al., 2006).

One of the many disease entities of PCVAD is PCV2-associated reproductive failure. Typically, increased numbers of abortions, mummified and stillborn fetuses and weakborn piglets are observed (O'Connor et al., 2001). To verify PCV2 as the causative agent, identification of microscopic lesions suggestive of fetal infection (myocardial fibrosis and lymphoplasmacytic myocarditis) and demonstration of PCV2 antigen in the heart lesions by immunohistochemistry (IHC) are used (Madson et al., 2009b). Piglet serum or fetal thoracic fluid may also be positive by PCR for PCV2 DNA or be positive for PCV2-specific antibodies (Madson et al., 2009b).

Vaccination against PCV2 has been shown to decrease losses due to PCVAD. There are currently several types of commercially vaccines on the market including: an inactivated PCV2a vaccine for usage in dams for protection of their piglets through colostrum-derived immunity or directly in piglets, two vaccines based on PCV2a-ORF2 expressed in baculovirus for usage in growing pigs, and an inactivated chimeric PCV2 vaccine also for usage in growing pigs which has been temporarily removed from the market as of May 2010. In addition to product differences, there are also different protocols for vaccine application, one dose or two dose (Gillespie et al., 2009).

All vaccines available today are inactivated or subunit vaccines. Another type of vaccine currently in the experimental stage of development is a live chimeric PCV2 vaccine. One concern with any live vaccine is the development of vaccine derived viremia in the pigs and spread of the vaccine virus between pigs and herds. It has been previously shown that a live chimeric PCV2 vaccine could prevent viremia and decrease macroscopic and microscopic lesions caused by PCV2 infection (Fenaux et al., 2004). Moreover, the pigs infected with PCV1-2a in Canada had no signs of clinical disease and there were no PCVAD outbreaks in the facilities where these animals came from (Gagnon et al., 2010).

PCV2 has been shown to be shed in oral, nasal and fecal excretions by several groups (Caprioli et al., 2006; Patterson et al., 2010; Segalés et al., 2005). Recently, it was demonstrated that PCV2 DNA can be found in all sex glands and shed in boar semen without damaging sperm morphology (Madson et al., 2008). It has also been shown that insemination of naïve dams with semen containing low levels of PCV2 DNA did not result in evidence of virus transmission (viremia, seroconversion) or reproductive failure (Madson et al., 2009c), however insemination with semen spiked with high levels of PCV2 DNA was capable of inducing reproductive failure in naïve dams (Madson et al., 2009b).

Objectives

- To determine if PCV2 vaccination using commercial PCV2 products in sows prior to breeding reduces or eliminates vertical transmission of PCV2 in the spiked semen model.
- To determine if there are differences between two commercially available one-dose products.
- To determine the safety of an experimental live PCV1-2 vaccine in conventional breeding animals.
- To determine the efficacy of an experimental live PCV1-2 vaccine in reducing and eliminating vertical transmission of PCV2 in the spiked semen model.

Materials and Methods

Animals and housing

Thirty-five sows, ranging in age from parity 0 to parity 7, were obtained from a herd confirmed to be free of PCV2. The sows were transported to Iowa State University in Ames, Iowa and kept in groups of 1 to 6 depending on room size. Each room was equipped with one nipple drinker and sows were fed daily with a pelleted feed ration free of animal proteins (excluding whey) and antibiotics (Nature's Made, Heartland Co-op, Cambridge, Iowa). The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee.

Experimental design

After arrival 12/35 sows were vaccinated with one dose (*1d*) of a live PCV2 vaccine (*1d-live-vaccine:PCV2*), 6/35 sows were vaccinated with 2 ml of an inactivated PCV2 vaccine once (*1d-vaccine:PCV2*), 6/35 sows were vaccinated with two 1 ml doses of the inactivated PCV2 vaccine three weeks apart (*2d-vaccine:PCV2*), and 11/35 sows remained unvaccinated as controls. Two weeks after the initial vaccination, estrous cycles were synchronized for all sows as previously described (Madson et al., 2009b). Three days after given PG600®, the sows were artificially inseminated. The extended semen was obtained from 9 boars, all of the same breed, confirmed to be PCV2 negative. Each sow in the negative control group and each sow in the live vaccinated, PCV2 negative group (*1d-live-vaccine*) received 80 ml of PCV2 free semen. All sows in the other groups were inseminated with 75 ml of semen spiked with 5 ml of PCV2b strain NC-16845 right before insemination. Inseminations were repeated in 24 h intervals for 3 days. The sows were bled upon vaccination weekly until necropsy at DPI 98.

Table 1. Experimental Design

Group	**Animals**	**PCV2 Vaccination (days prior to breeding)**		**PCV2 inoculation**
		35	**14**	
Negative Control	5	-	-	-
Positive Control	6	-	-	Spiked semen
2d-vaccine:PCV2	6	YES	YES	Spiked semen
1d-vaccine:PCV2	6	YES	-	Spiked semen
1d-live-vaccine:PCV2	6	YES	-	Spiked semen
1d-live-vaccine	6	YES	-	-

Clinical observation

All sows were examined daily for signs of illness such as: lethargy, respiratory disease, inappetence and lameness.

Inoculation

Five weeks after arrival and vaccination, semen was spiked with 5 ml PCV2b and used to artificially inseminate 24/35 sows. The PCV2b isolate NC-16845 (Opriessnig et al., 2008) used for the inoculation was propagated to an infectious titer of $10^{4.5}$ 50% tissue culture infective dose ($TCID_{50}$).

Sample collection

Blood was collected from sows in 8.5 ml serum separator tubes (BD vacutainer®, BD Biosciences) on a weekly basis until necropsy. At necropsy, pre-suckle blood was collected from all live-born fetuses and fetal thoracic fluid was collected from all still-born or mummified fetuses if possible. The blood was centrifuged at 4000 rpm for 10 min at 4ºC and the serum was aliquoted into 5 ml polystyrene round bottom tubes (Fisher Scientific, Inc.) and stored at -20ºC until testing.

Serology

All serum samples were tested for PCV2-specific IgG antibodies using an indirect PCV2 ORF2 based ELISA as previously described (Nawagitgul et al., 2002). The results were expressed as sample-to-positive (S/P) ratio. Samples were considered to be negative if the S/P ratio was less than 0.2 and positive if the S/P ratio was greater than or equal to 0.2.

PCV2 viremia detection

PCV2 DNA detection was conducted to verify the presence of PCV2 in all serum, fetal thoracic fluid, and tissue samples. PCV2 viral DNA was extracted from the samples using the MagMax^{TM} Viral RNA Isolation Kit (Applied Biosystems, by life technologies, Carlsbad, California) on the KingFisher Flex System (ThermoFisher Scientific, Pittsburgh, PA). PCV2 DNA detection to verify and quantify the presence of PCV2 in all serum samples was done by a quantitative real-time PCR using the same primers and probes as previously described (Opriessnig et al., 2003) using the TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, Foster City, California, USA). The total PCR reaction volume, including 2.5 µl of DNA extract was 25 µl. The thermal cycle conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. Samples were considered negative if no signal was observed during the 40 amplification cycles.

PCV1-2 PCR

PCV1-2 DNA detection was done to verify the presence of any PCV1-2 using the same samples as the PCV2 PCR. DNA quantification was done by quantitative real-time PCR using the same primers and probes as previously described (Shen et al., 2010). The total PCR reaction volume was 25 µl. The thermal cycle conditions were 50ºC for 2 min, 95ºC for 10 min, followed by 40 cycles of 95ºC for 15 sec, 55ºC for 30 sec, and 60ºC for 1 min. Samples were considered negative if no signal was observed during the 40 amplification cycles.

Necropsy

All sows were humanely euthanized by intravenous pentobarbital sodium overdose (Fatal Plus ®, Vortech Pharmaceuticals, LTD, Dearborn, Michigan) and necropsied at 105 days of gestation. Presuckle blood was collected from all live-born piglets and the piglets were then humanely euthanized by intravenous pentobarbital sodium overdose (Vortech Pharmaceuticals, LTD) and necropsied. If blood collection was not possible (mummified fetuses or stillborns), fetal thoracic fluid was collected whenever available. Fetal heart tissues were collected at necropsy, a section stored fresh at -20ºC until further testing and a section fixed in 10% neutral buffered formalin. Fixed tissues were then routinely processed for histological examination, embedded in paraffin and stained with hematoxylin and eosin as previously described (Halbur et al., 1995).

Immunohistochemistry

Immunohistochemistry (IHC) for detection of PCV2-specific antigen was performed on fetal hearts using a rabbit polyclonal antiserum (Sorden et al., 1999). Antigen scoring was performed by a veterinary pathologist blinded to treatment groups and scores were reported from 0 (no antigen detected) to 3 (more than 50 percent of cells contained PCV2 antigen) as previously described (Opriessnig et al., 2004).

Statistical analysis

Statistical analysis of the data was performed using the JMP® software version 9.0.0 (SAS Institute, Cary, NC). Summary statistics were calculated for all groups to assess the overall quality of the data including normality. One-way analysis of variance (ANOVA) was used to evaluate the differences among treatment groups. If differences in group means were observed then Tukey-Kramer test was used for each pair-wise comparison. 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) and FFN results were $\log_{10}$ transformed prior to statistical analysis. A non-parametric ANOVA (Kruskal-Wallis) was used for non-normally distributed data or when group variances were dissimilar.

Results

Reproductive parameters

All dams tested negative for PRRSV and PPV prior to initiation of the study. Thirty-one of 35 sows were not pregnant at necropsy. Of the four pregnant sows, the litter compositions were as follows: 2 mummies and 12 liveborn piglets (one live chimeric PCV2 vaccinated sow), 4 mummies and 11 liveborn piglets (one inactivated chimeric PCV2 vaccinated sow), 0 mummies and 10 liveborn pigets (one negative control sow) and 1 mummy and 13 liveborn piglets (one positive control sow).

Clinical observation

One negative control sow and one *1d-live-vaccine:PCV2* sow developed severe lameness and were euthanized at DPI 84.

Seroconversion to PCV2

Negative control sows remained negative for PCV2-specific antibodies throughout the study. All *1d-vaccine:PCV2* and *2d-vaccine:PCV2* sows had seroconverted prior to the first serum collection at DPI -7. Five/6 1d-live-vaccine and 5/6 *1d-live-vaccine:PCV2* sows had seroconverted by DPI 7. There was no significant difference in antibody levels between the *1d-vaccine:PCV2* and *2d-vaccine:PCV2* groups except at DPI -7. Also, there was no significant difference in antibody levels between the *1d-live-vaccine* and *1d-live-vaccine:PCV2* groups. Sows vaccinated with the inactivated vaccine had significantly higher ($P<0.05$) antibody levels detected by ELISA than those inseminated with the live vaccine every DPI throughout the course of the study. All fetuses from all 4 litters were negative for PCV2-specific antibodies at birth.

PCV2 viremia

Negative control sows as well as the *1d-live-vaccine* sows remained negative for PCV2 viremia throughout the study. Positive control sows became positive for PCV2 viremia at DPI 14 and remained positive for the remainder of the study. All groups of challenged, vaccinated sows became viremic at varying DPIs during the study; however, there was no significant difference in viremia across groups at any time point (Table 2). Positive control piglets had significantly higher ($p<0.05$) levels of PCV2 DNA detected at birth when compared to the vaccinated and negative control piglets (Table 3).

PCV1-2 viremia

All sows remained negative for PCV1-2 viremia throughout the course of the study (Table 2). Three of 14 fetuses from the live PCV1-2 vaccinated group were positive PCV1-2 DNA (Table 3) with an average $\log_{10}$ transformed mean amount PCV1-2 DNA of 0.82±0.44 per ml.

Table 2. Number of sows positive by PCR for PCV2 and PCV1-2 DNA per group.

Group	-7	7	14	21	28	35	42	49	56	77	84	91	98	105
1 dose	0/6	0/6	0/6	1/6	0/6	1/6	0/6	1/6	0/6	0/6	0/6	1/6	0/6	0/5~
2 dose	0/6	0/6	0/6	1/6	0/6	0/6	0/6	1/6	0/6	1/6	0/6	0/6	1/6	0/5~
1-2 neg	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5~
1-2 pos	0/6	0/6	1/6	1/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5*	0/5*	2/5*

*One PCV1-2 live vaccinated, PCV2 challenged sow developed severe lameness and was euthanized at DPI 84.
~One FDAH 1 dose sow, one FDAH 2 dose sow, and one PCV1-2 live vaccinated, unchallenged sow were necropsied on DPI 102.

Table 3. Number of piglets positive by PCR for PCV2 and PCV1-2 DNA per group.

DNA detected	Group	Necropsy
PCV2	1-2 pos	4/14
	2 dose	9/15
	Neg	0/10
	Pos	13/13
PCV1-2	1-2 pos	3/14
	2 dose	0/15
	Neg	0/10
	Pos	0/13

PCV2 antigen in tissues

All 10 piglets born to the negative control sow and all 29 piglets from the vaccinated sows were negative for PCV2 antigen on heart tissues. Low-to-abundant amounts of PCV2 antigen were detected in 3 of 13 fetuses from the non-vaccinated positive sow.

Discussion

When intranasally infected with PCV2, sows become viremic and transplacental transmission of PCV2 occurs (Park et al., 2005). It has also been shown that sows can become viremic when inseminated with PCV2 positive semen (Madson et al., 2009b) and even vaccinated sows are capable of producing PCV2 positive piglets (Madson et al., 2009a). These viremic piglets could be an important source of PCV2 transmission in farrowing barns. In this current study, sows were inseminated with PCV2 spiked semen, and viremia was detected in the positive control group confirming that PCV2 can be spread through semen. PCV2 positive piglets were also detected in all challenged sows confirming that even vaccinated sows are capable of producing PCV2 positive piglets.

Currently there are two vaccines on the market approved for use in healthy pigs 3 weeks or older and 1 vaccine approved for use in healthy breeding age females. Vaccination of sows and/or piglets has been shown to decrease mortality rates and controlling PCVAD (Kixmöller et al., 2008; Pejsak et al., 2010). Results from the current study indicate that the use of three different vaccination protocols all decreased PCV2 viremia and increased anti-PCV2-specific antibody production when compared to the unvaccinated control group. Interestingly, the *1d-vaccine:PCV2* and *2d-vaccine:PCV2* groups developed higher levels of antibodies at all DPI's tested except at the time of necropsy compared to the *1d-live vaccine* and *1d-live vaccine:PCV2* groups. The use of two different vaccines (the live chimeric PCV1-2 vaccine and the 2 dose inactivated chimeric PCV1-2 vaccine) resulted in a decrease in PCV2 DNA detection in piglets when compared to the positive control group; however, the vaccines failed to fully protect against reproductive failure. This could be due to only a few sows becoming pregnant in this study. In a previous study comparing several commercial and experimental vaccines in young pigs, there was no significant difference between any of the vaccine groups (Shen et al., 2010), in contrast to the results found here.

In Canada recently, PCV1-2 virus was isolated from 3 different pigs in herds that vaccinated with a PCV1-2 chimeric inactivated vaccine (Gagnon et al., 2010). This raises the question of whether PCV1-2 virus could cause adverse effects in pigs. Several studies have shown that the PCV1-2 clones and the attenuated live PCV1-2 vaccine are capable of inducing an antibody response but are attenuated in pigs and not capable of producing the characteristic lesions of PCV2 infection (Fenaux et al., 2003; Fenaux et al., 2004; Gillespie et al., 2008). In this study, the *1d-live vaccine* group had no evidence of viremia; however, they did develop a PCV2-specific antibody response. The *1d-live vaccine:PCV2* group developed viremia at a few time points at low levels which quickly resolved and piglets from this group had low levels of PCV1-2 viremia at birth. Despite the viremia, no lesions were noted and no PCV2 antigen was found in heart tissues of fetuses further confirming results previously found further demonstrating that the vaccine is capable of inducing the antibody response and is attenuated.

In conclusion, there were no significant differences in viremia of sows between vaccinated groups, however the inactivated vaccine was capable of producing higher levels of antibodies. All piglets from vaccinated sows had statistically lower levels of PCV2 DNA than the positive control group indicating they were successful at decreasing viremia. Interestingly, though no PCV1-2 DNA was detected in sows, PCV1-2 DNA was found in piglets from the live chimeric PCV1-2 vaccinated sow. Despite the viremia, no antigen or lesions were found indicating that all vaccines used are safe for use in breeding age dams.

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