Efficacy and impact of current commercial porcine circovirus type 2 (PCV2) vaccines in dams and growing pigs

Kevin Charles O'Neill
Iowa State University

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Efficacy and impact of current commercial porcine circovirus type 2 (PCV2) vaccines in dams and growing pigs

By

Kevin C. O’Neill

A thesis submitted to the graduate college in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

Major: Immunobiology

Program of Study Committee:

Tanja Opriessnig – Major Professor
Bradley Blitvich
Patrick Halbur
Annette O’Connor

Iowa State University
Ames, Iowa
2012

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CHAPTER 1 - INTRODUCTION

1. Introduction

Porcine circovirus (PCV) was initially described as a contaminant of the continuous porcine kidney cell line PK-15 in 1974 (Tischer et al., 1974). It is a covalently closed single-stranded DNA virus belonging to the genus Circovirus in the family Circoviridae (Tischer et al., 1982). In early studies it was determined that PCV is ubiquitous, but non-pathogenic (Tischer et al., 1986). In 1991, a severe wasting disorder was observed in pigs shortly after weaning in western Canada (Harding et al., 1997; Clark, 1997), the United States (Allan et al., 1998b), and Europe (LeCann et al., 1997; Allan et al., 1998b). Although PCV was identified in tissues from affected pigs, it was determined to differ from the original PCV genome by 24% to 32% (Meehan et al., 1998; Cheung et al., 2007). This led to classification of PCV into two distinct genotypes: the original non-pathogenic strain or PCV type 1 (PCV1) and the newly isolated, pathogenic strain or PCV type 2 (PCV2) (Meehan et al., 1998). Further genomic alignments revealed that PCV2 could be separated into two distinct groups: group 1 comprised of three distinct clusters (1A to 1C), and group 2 comprised of five distinct clusters (2A to 2E) (Olvera et al., 2007). Due to multiple designations for these groups (de Boisseson C. et al., 2004; Olvera et al., 2007; Timmusk et al., 2008; Grau-Roma et al., 2008; Carman et al., 2008), use of lower case letter designations for each group was proposed by Segalés et al. (2008) for uniformity, resulting PCV2 group 1 becoming PCV2a and PCV2 group 2 becoming PCV2b (Segalés et al., 2008).

Since its original identification as the etiological agent of postweaning multisystemic wasting syndrome (PMWS) (Ellis et al., 1998; Meehan et al., 1998; Allan et al., 1998a),
PCV2 has been associated with multiple disease manifestations, collectively referred to as PCV-associated disease (PCVAD) (Opriessnig et al., 2007). Besides PMWS (Clark, 1997; Harding et al., 1998), these disease manifestations include respiratory disease (Kim et al., 2003), enteric disease (Kim et al., 2004), reproductive failure (West et al., 1999), and porcine dermatitis and nephropathy syndrome (PDNS) (Choi et al., 2001). All are considered of high economic importance due to their contribution to ill-thriftiness, morbidity rates varying between 12.5% and 59% (USDA, 2008), and variable mortality rates (López-Soria et al., 2005; Calsamiglia et al., 2007; Alarcon et al., 2011; Grau-Roma et al., 2012).

PCV2 is considered ubiquitous and is prevalent globally (Tischer et al., 1986; Dulac et al., 1989; Edwards et al., 1994; Allan et al., 2000; Walker et al., 2000; Zhou et al., 2006; Segalés et al., 2008) due to both its resilience to inactivation methods (Allan et al., 1994; Welch et al., 2006; O'Dea et al., 2008) and its numerous methods of transmission. These include horizontal transmission routes such as colostrum (Shibata et al., 2006), feces (Shibata et al., 2003), invertebrate vectors (Blunt et al., 2011), nasal secretions (Shibata et al., 2003), oral secretions (Shibata et al., 2003), and seminal fluids (Larochelle et al., 2000). Vertical transmission routes include either early crossing of the zona pellucida resulting in oocyte infection (Bielanski et al., 2004; Mateusen et al., 2004) or intrauterine fetal infection during dam viremia (West et al., 1999; O'Connor et al., 2001; Ladekjær-Mikkelsen et al., 2001; Nielsen et al., 2004; Park et al., 2005; Madson et al., 2009b).

In response to a large outbreak of PCVAD in late 2005 (Cheung et al., 2007), which coincided with a global shift of PCV2a towards PCV2b (Cheung et al., 2007; Gagnon et al., 2007; Olvera et al., 2007; Segalés et al., 2008), PCV2 vaccines were introduced on the North American market in 2006. To date, there are five commercially available vaccines, four of
which are licensed for use in the United States by the United States Department of Agriculture. These vaccines use three different methods of antigen delivery, all are licensed for use in weaning age pigs, and one is additionally licensed for use in breeding dams. The first developed vaccine, Circovac® (Merial Inc.), was experimentally launched in 2004 (Reynaud et al., 2004a, 2004b) and was initially only available in France and Germany under special license (Charreyre et al., 2005), but was globally licensed in late 2007 (Burch, 2008). Circovac® is based on an inactivated PCV2a, and is unique that it is licensed for both weaning age piglets and breeding dams. It is currently licensed for use in the US, but is not commercially available. Fostera™ PCV (Pfizer Animal Health Inc.) is a reformulated version of Suvaxyn® PCV (Fort Dodge Animal Health Inc.), and was launched in North America in 2011 after Suvaxyn® PCV was voluntarily removed from the market in May 2010 in response to concerns of improper inactivation (Gagnon et al., 2010). Fostera™ PCV is based on an inactivated chimeric virus in which the capsid gene of PCV2a is cloned into the non-pathogenic backbone of PCV1 (Fenaux et al., 2004) which has been demonstrated to be attenuated in vivo (Fenaux et al., 2003). Fostera™ PCV is used as a single dose in weaning age pigs. The remaining three vaccines are all based on ORF2 protein expressed in a baculovirus vector system (Beach et al., 2012). Ingelvac® CircoFLEX™ (Boehringer Ingelheim Vetmedica Inc.) is administered as a single dose to weaning age pigs while Circumvent® PCV (Merck Inc.) is administered as two doses at weaning and two weeks later. Both vaccines are licensed for use and commercially available in the US. Porcilis® PCV (MSD Inc.) is administered as a single dose at weaning and is not currently available in the US.
Within the PCV2 literature, there have only been two peer-reviewed meta-analyses; one on experimental trials with PCV2 (Tomás et al., 2008) and the other on general efficacy of PCV2 vaccines (Kristensen et al., 2011). Furthermore, there have also been three meta-analyses presented at conferences, which focused solely on the performance of Ingelvac® CircoFLEX™ (Holck et al., 2010; Diaz et al., 2010; Coll et al., 2010). Thus, our first study, a pairwise meta-analysis of the commercially available PCV2 vaccines in the United States, was performed using previous literature through a critical review and comparing the efficacy of the vaccines compared to no vaccine administration.

In the US, PCV2 vaccination is currently only licensed for use in pigs at 21 days of age or older. However, producers commonly vaccinate piglets against PCV2 during routine processing of piglets, including teeth clipping, tail docking, castration, and iron administration which occurs within the first few days of life (Marchant-Forde et al., 2009), in an attempt to minimize handling and stress. Thus, the main objective of our second study was to compare two commercially available vaccines, and their efficacy in 5-day-old and 21-day-old piglets.

Recently, much attention has been given to the role of dam vaccination and vertical transmission of PCV2 (Madson et al., 2009a; Madson et al., 2009c; Madson et al., 2011; Kurmann et al., 2011). Furthermore, a recent field study across North America demonstrated a 44.8% (226/504) prevalence of PCV2 viremia in neonatal piglets with a large portion of the piglets appearing normal and healthy (Shen et al., 2010). To further characterize the role of dam PCV2 vaccination in newborn piglet viremia, our third study focused on extra-label use of a commercial available piglet vaccine in breeding females. Randomly selected dams were vaccinated with the PCV2 vaccine prior to insemination or left unvaccinated. At parturition,
blood samples were collected from five healthy, randomly-selected piglets from each litter and colostrum was collected from the dam to determine if vaccination of the dam reduces the presence of PCV2 in the colostrum and the offspring.

2. Thesis organization

This thesis has been organized using the alternate manuscript format, containing an introduction, three separate chapters, and a conclusion. Two of the three chapters are scientific manuscripts. References for the introduction and conclusion are cited at the end of this document in the "References" section. References for all other chapters are cited at the end of each chapter.

The first chapter is the introduction to the topic. The second chapter serves as a literature review in the form of a pairwise meta-analysis. The third chapter is a scientific manuscript on efficacy of two commercially available vaccines in 5-day-old and 21-day-old piglets, published in Clinical and Vaccine Immunology (O'Neill et al., 2011). The fourth chapter is a scientific manuscript on the effect of dam vaccination on offspring PCV2 viremia. This manuscript is published in the Veterinary Record (O'Neill et al., 2012). The fifth and final chapter is a summary of the conclusions presented within this thesis.
CHAPTER 2 – CRITICAL REVIEW AND PAIRWISE META-ANALYSIS OF THE THREE COMMERCIALLY AVAILABLE PCV2 VACCINES IN THE UNITED STATES.

1. Abstract

Porcine circovirus type 2 (PCV2) vaccines have become one of the most frequently administered vaccines to growing pigs worldwide since they became commercially available in 2006. They are generally considered to be very effective. While there are multiple studies on the efficacy of a singular PCV2 vaccine, there are few studies investigating the overall effect PCV2 vaccines on the swine industry.

Objective: To perform a pairwise meta-analysis of the three commercially available PCV2 vaccines in the United States.

Data sources: A literature search for relevant studies using three major databases (PubMed, CAB Abstracts, and AGRICOLA) and the proceedings of the American Association of Swine Veterinarians Conference, Allen D. Leman Swine Conference, Iowa State University Swine Disease Conference for Swine Practitioners, and the International Pig Veterinary Society Congress was conducted. Additionally, a search of the United States Department of Agriculture (USDA) Center for Veterinary Biologics (CVB) licenses and provisions was performed.

Study selection: Only randomized field studies of the three commercially available, USDA licensed PCV2 vaccines administered according to manufacturers’ specifications were included. The outcome of interest was the average daily gain (ADG) from wean to finish.
Data extraction: Independent extraction of studies by two researchers was performed using pre-defined parameters for data, including risk bias analysis. These data were then used in a pairwise meta-analysis.

Data synthesis: Pigs vaccinated with Ingelvac® CircoFLEX™ (13 trials, n=5,417) experienced a greater ADG, with a mean difference of 27.84 g/day (95% CI, 20.06 – 35.63) over non-vaccinated pigs in the same trials (n=5,078). Pigs vaccinated with Circumvent® PCV (6 trials, n=587) also had a greater ADG (mean difference=39.45; 95% CI, 31.27 – 47.63) than their non-vaccinated counterparts (n=544). Interestingly, pigs vaccinated with either Suvaxyn® PCV2 or Fostera™ PCV (2 trials, n=385) had a greater, though not significant, ADG (mean difference=44.76; 95% CI, -14.32 – 103.84) when compared to non-vaccinated pigs (n=375). Furthermore, there was no difference in ADG between studies in which the herds were porcine reproductive and respiratory syndrome virus (PRRSV) positive or PRRSV negative.

Conclusions: All products reviewed in this pairwise meta-analysis demonstrated increases in ADG, though only Ingelvac® CircoFLEX™ and Circumvent® PCV demonstrated significant differences between vaccinated and non-vaccinated animals. Likely, the small numbers of studies available for Suvaxyn® PCV2/Fostera™ PCV limited its applicability to the pairwise meta-analysis.

2. Introduction

Vaccination against porcine circovirus (PCV) type 2 (PCV2) is a common method to protect growing pigs against the clinical disease manifestations associated with PCV2, commonly referred to as porcine circovirus associated disease (PCVAD) (Opriessnig et al.,
Vaccines against PCV2 were developed, licensed, and initially distributed in the United States in 2006 in response to a severe outbreak of PCVAD in North America in 2005 and 2006 (Cheung et al., 2007). There are indications from the field that PCV2 vaccines are efficacious at preventing disease and increasing production parameters (Kixmøller et al., 2008; Fachinger et al., 2008; Gillespie et al., 2009).

To date, there are three commercial vaccines licensed and available for use in pigs three weeks of age and older in the United States. Fostera™ PCV (Pfizer Animal Health, New York, NY), a reformulated version of Suvaxyn® PCV (Fort Dodge Animal Health, Fort Dodge, IA), uses an inactivated chimeric PCV1-2 virus, in which the capsid gene of PCV2a is cloned into the non-pathogenic PCV type 1 (PCV1) backbone. This vaccine is administered in a single dose. Ingelvac® CircoFLEX™ (Boehringer Ingelheim Vetmedica, St. Joseph, MO) and Circumvent® PCV (Merck Animal Health, Omaha, NE) both are based on the PCV2a capsid gene expressed in a baculovirus vector. CircoFLEX® is delivered as a single dose, while Circumvent® PCV is administered in two doses three weeks apart from each other. Currently, available product efficacy data are limited to studies utilizing an individual product, and comparison data of efficacy among vaccines are limited.

We are aware of only one peer-reviewed meta-analysis summarizing the effect of commercially available PCV2 vaccines (Kristensen et al., 2011). The current review updates that meta-analysis and makes greater use of data derived estimates of variation of the outcome ADG. The prior meta-analysis used the inverse of the study size to describe within-study variation of ADG and to calculate the summary effect measure. Such an approach is not ideal as it assumes that variation in the study population is a direction function of sample size whereas in reality, variation in the ADG in pigs may be a function of many factors. In
this review we incorporated variation estimates from each study where possible. Narrative reviews serve a great purpose in assembling and summarizing current knowledge, but systematic reviews allow for in-depth inspection of collective data using a concentrated study question, reproducible search and extraction methods, and bias analysis. The data obtained through the systematic review process can then be easily adapted for use in an overall meta-analysis.

The objective of this study was to summarize an estimate of efficacy and impact of commercially available PCV2 vaccines when used in intensively raised swine. In this review we conducted a pairwise meta-analysis of the three commercially available PCV2 vaccines in the US, relative to no vaccine.

3. Materials and Methods

1. Protocol and registration.

A review protocol was developed using Review Manager 5.1 (RevMan 5.1, Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). The search strategy was modified after the protocol was established based on suggestions from experts (D. Holtkamp, P. Halbur) outside the review team (K. O’Neill, A. O’Connor, T. Opriessnig). Also, due to anticipated difficulties with the data analysis, the protocol only proposed mixed treatment comparison (MTC) meta-analysis and did not specify the exact method of data analysis. This paper describes a pairwise meta-analysis of each vaccine compared to a non-active control arm as an intermediary step in the review, prior to conducting the MTC. The rationale for conducting a pairwise meta-analysis prior to an MTC is to enable verification of the data and establish exceptions for associations prior to conducting an MTC.
II. Eligibility criteria

The PICOS-based question (P=population; I=intervention; C=comparator; O=outcome; S=study design) for the critical review (Sargeant et al., 2006) was developed in consultation with a veterinary epidemiologist (A. O’Connor) and a veterinary pathologist (T. Opriessnig). The final question agreed upon was “What is the effect of each of the three commercially available PCV2 vaccines compared to no vaccines on average daily gain between weaning and marketing in commercial pigs naturally exposed to PCV2 where the porcine reproductive and respiratory syndrome virus (PRRSV) status is known?” Within the PICOS format of the question development, the population of interest was defined as intensively raised pigs in a modern, commercial setting. The intervention was defined as any United States Department of Agriculture (USDA) licensed, commercial PCV2 vaccine available in the US administered as prescribed by the manufacturer, though its use was not geographically limited. The comparator of interest was defined as a non-active arm such as no vaccine, saline, etc. The outcome was defined as average daily gain (ADG) (g/day) from wean to finish. The study design of interest was randomized trials with naturally occurring PCV2 exposure and disease, i.e. experimental studies were excluded.

III. Information sources

Studies relevant to the review were chronologically self-limited as vaccines have only been commercially available since 2006. Therefore the search data for relevant studies were limited to a beginning date of January 1st, 2006, and to publication in English, Spanish, and Portuguese due to scientific staff fluency and lack of reliable translation software.
A subject librarian specializing in veterinary medicine (A. Dinkelman) was consulted for the development of a Boolean search string for an electronic database search of PubMed (http://www.ncbi.nlm.nih.org/pubmed/), AGRICOLA (EBSCOhost® Research Database, EBSCO® Publishing, Ipswich, MA), and CAB Abstracts (Web of Knowledge™, Thomson Reuters, New York, NY). An additional limit was to adjust the search engine for articles pertaining to animals. Searches were performed on April 27th, 2012, May 1st, 2012, and May 8th, 2012. On May 17th, 2012 a non-Boolean search of the USDA Center for Veterinary Biologics (CVB) was performed to both confirm the current product licensure for the United States (USDA, 2012) and to potentially find the required studies for licensure (USDA, 2011; USDA, 2012).

A search of the Swine Information Library (http://www.aasv.org/library/swineinfo/) through the proceedings of the International Pig Veterinary Society (IPVS) Congress, the American Association of Swine Veterinarians (AASV) conference, the Allen D. Leman Swine Conference, and the Iowa State University (ISU) Swine Disease Conference for Swine Practitioners from 2006 to 2012 was also performed between August 28th, 2012 and August 30th, 2012.

IV. Example search

Individual parameters directly applicable to the PICOS-formulated question were chosen with the aid of the subject librarian and were entered into the following search string with Boolean operators: “population of interest” AND “outcome” AND “intervention” (Table 1). The PubMed search string was as follows: (Barrow OR Barrows OR Boar OR Boars OR Feeder OR Finishing OR Gilt OR Gilts OR Hog OR Hogs OR Pig OR Piglet OR
Piglets OR Pigs OR Porcine OR Shoats OR Sow OR Sows OR Swine) AND ("Acute Pulmonary Edema" OR “Acute Pulmonary Oedema” OR Circoviridae OR Circovirus OR PCV OR PCV2 OR PCV-2 OR PCVAD OR PCVD OR PMWS OR “Porcine circovirus” OR “Porcine circovirus 2” OR “Porcine circovirus associated disease” OR “Porcine circovirus disease” OR “Porcine circovirus type 2” OR “Porcine circovirus-2” OR “Porcine respiratory disease complex” OR “Postweaning multisystemic wasting disorder” OR “Porcine Postweaning Multisystemic Wasting Syndrome [MeSH]” OR PRDC) AND (Immunisation OR Immunise OR Immunised OR Immunity OR Immunization OR Immunize OR Immunized OR Immunoprophylaxis OR Intervention [tiab] OR Interventions [tiab] OR Vaccinate OR Vaccinated OR Vaccination OR Vaccinations OR Vaccine OR Vaccines). The only restrictions imposed were chronological limitation of the publication dates spanning 2006/01/01 to 2012/05/01 and setting the species from Humans to Other Animals.

All citations retrieved were imported into a reference managing database (Reference Manager® ver. 11, Thomson Reuters) in which duplicates were eliminated, either through software detection or manual review.

V. Study selection process

In this review, “study” was considered a clinical trial which fulfilled the eligibility criteria and there could be multiple “studies” per article. Once records were retrieved they were evaluated for eligibility based on the following screening questions: 1) Does the study describe an assessment of one of the three listed commercially available PCV2 vaccines within a field trial with a natural exposure? 2) Does the study report both the vaccine and its administration in accordance with the manufacturers’ specifications? 3) Does the study
report ADG (wean to finish)? 4) Is the PRRSV status of the herd included? These questions were evaluated using five abstracts by three individuals (A. O’Connor, T. Opriessnig, K. O’Neill) and modified to ensure they selected the correct studies. After this step screening of the remaining citations was conducted by only one person (K. O’Neill).

The screening process was conservative, i.e., a record was considered relevant until found not to be. Initial screening of a study for relevance was performed at the title level, where ambiguous titles containing any combination of terms from the Boolean search string were conservatively considered relevant, unless they explicitly described a trial that utilized a vaccine other than the three licensed for commercial use in the US. Further review of each study, first through the abstract, then, if not clearly defined there, the full text, was based on the above questions.

Any negative response to the first three of these questions deemed the study ineligible. Authors of otherwise qualifying studies were contacted via e-mail to identify the PRRSV status of the herd within the study. Failure to identify the PRRSV status of the herd, through either extraction or electronic contact meant the study was outside of the scope of the review. The rationale of this eligibility criteria was that a previous meta-analysis of general PCV2 vaccine efficacy (Kristensen et al., 2011) regarded PRRSV status as a potential source of clinical variation and excluded studies if PRRSV was not identified as being absent or present in the herd, although PRRSV status classification was not addressed (Kristensen et al., 2011). Furthermore, previously reported high incidences of disease manifestations associated with concurrent PCV2 and PRRSV (Pallarés et al., 2002; Wellenberg et al., 2004; Grau-Roma et al., 2007) indicate a substantial role of PRRSV in the pathogenicity of PCV2.
VI. Data collection process

Once a study was considered relevant, data available in English, Spanish, and Portuguese were extracted by two Master level students (K. O’Neill, P. Gerber), specialized in PCV2 research, working independently. The forms for data extraction were not pretested or piloted. Conflicts between the two researchers were identified and resolved by one reviewer (K. O’Neill). After data extraction was completed, five randomly selected relevant papers were sent to an independent reviewer, unconnected to the project (R. Dzika) to verify the extraction of outcome data. The independent reviewer did not assess demographic or bias data extraction. The results of that exercise identified disagreements for three of the five studies, which were resolved through discussion. The primary reviewer (K. O’Neill) then modified the output presentation for all extracted data after that discussion to reduce errors.

4. Data items collected

1. Outcome data

For the primary outcome, ADG in g/day from weaning (approximately three weeks of age) to finish (approximately 23 weeks of age) data were required for each arm of each trial. Outcome data extracted included ADG (wean to finish) for each arm, the sample size for each group, measures of variation i.e. SD, SEM or p-values when reported.

The units for the primary summary measure of ADG were converted to grams per day for all studies. In some instances studies did not report ADG for the required period from weaning to finish. In these cases, although it seemed likely that the study would have generated the desired information, the study was not incorporated into the meta-analysis. On several occasions, the data could be extracted with manipulation and was done by combining
either multiple groups (i.e. the original study report listed the data stratified by sex) or
production stage time measurements (i.e. ADG with measures of variation were reported
separate for nursery, grow-finish, and finish stages for a single group).

Some studies reported weight measurements and measures of variation for all
necessary time points, but the authors did not calculate or clearly report ADG. In these
instances, the ADG was calculated using the equation

\[
\frac{\text{Final Weight Reported} - \text{Initial Weight Reported}}{\# \text{ of days in production}}
\]

and then the reported measures of variation were conservatively calculated using

\[
\frac{SD_{\text{Initial Weight Reported}} + SD_{\text{Final Weight Reported}}}{\# \text{ of days in production}}
\].

To calculate overall ADG from an individual herd in which the data was reported in
separate production stages, the following equation was incorporated:

\[
\frac{(ADG_1 \times \# \text{ of days in stage}_1) + (ADG_2 \times \# \text{ of days in stage}_2)}{\# \text{ of days in stage}_1 + \# \text{ of days in stage}_2}
\].

To account for inconsistent measure of variation for the ADG reporting, the standard
deviation (SD) of each treatment group mean was derived when not reported. Meta-analysis
requires ADG and SD for each study group, and if this information was not reported we used
wherever possible the RevMan 5.1 statistical calculator (RevMan) to calculate the SD for
each treatment group mean. When reporting was incomplete, such as authors reported a
probability value (p-value) only, back-calculation was conducted to determine the measures
of variation. However, if the number of animals enrolled and the ADG were not reported it
was not possible to extrapolate the needed numbers, and although relevant to the review the
studies’ results could not be included in the meta-analysis.

If a p-value was reported, the p-value, mean difference, and population were used to
derive a T-statistic (RevMan). The T-statistic was then used in the equation

\[
\frac{\text{Mean difference}}{T \text{-statistic}} =
\]
SE_{Mean\ Difference}. The derived SE_{Mean\ Difference} was entered in the RevMan 5.1 statistical calculator (RevMan) with the p-value, mean difference, and population data points to obtain the SD for each mean. Furthermore, the p-value was conservatively used in these instances, i.e. for studies that reported p<0.05 we used p=0.05 in our calculations.

When ADG was reported within a single herd but reported in various production stages, the measures of variation generally had to be combined similarly. This resulted in a conservative estimation of the overall SD using the following equation:

\[
\frac{(# \ of \ days \ in \ stage_1 \times SD_{Initial\ Weight\ Reported}) + (# \ of \ days \ in \ stage_2 \times SD_{Final\ Weight\ Reported})}{# \ of \ days \ in \ stage_1 + # \ of \ days \ in \ stage_2}.
\]

II. Intervention, and clinical and methodological sources of heterogeneity

Information about the interventions was also extracted i.e. the PCV2 vaccine used, timing, and control treatments. Information collected from the studies included the sources of clinical and methodological heterogeneity and the outcome of interest and vaccination age. Extracted information about potential sources of clinical heterogeneity included variables such as location (country) and PRRSV status (negative or positive). Extracted methodological sources of variation included the unit of treatment allocation (pig/piglet/sow, pen, barn, etc.), method of allocation to treatment group i.e. did the authors report randomization (yes or no), and whether blinding of the outcome assessor to the treatment group status was reported (yes or no). Information about allocation concealment and methods of sequence generation were not extracted.

Studies which used pen and barn level allocations were reviewed to determine if treatment group integrity was maintained, which could bias the estimates of ADG (Van
Vleck et al., 2005). To account for this, pen effects were entered in the model as random effects (Chen et al., 2008).

To avoid over-estimation of ADG, only studies which accounted for mortality in ADG calculation (Booker et al., 2007) were determined eligible. When ADG needed to be calculated, mortality was accounted for, and only pigs that survived until the finishing phase were included.

During the data collection process, investigators were contacted via e-mail for clarification of certain data, such as PRRSV status of the herd, measures of variation, and methods of allocation to group such as blocking and randomization.

There is a clearly documented difference of PCV2-associated microscopic lesions (lymphoid depletion and granulomatous inflammation) in purebred Landrace pigs compared to Pietrain, Large White, and Duroc pigs (Opriessnig et al., 2006a; Opriessnig et al., 2009d), and breed genetics have accounted for differences in susceptibility to disease from other viral infections such as PRRSV (Halbur et al., 1998) and pseudorabies virus (Reiner et al., 2002). While this could introduce bias to this study, crossbreeding is typically utilized by pork producers to optimize production (Bennett et al., 1983). If the herd breed was disclosed, it was documented, but the assumption to treat all breeds equally in this study was made due to the animals in question housed in intensive swine production units which utilize crossbreeding (Kuhlers et al., 1994).

III. Risk of bias in individual studies

The methods of randomization and blinding as part of the Data items in the Data collection process were evaluated for a potential bias using the Cochrane risk bias
assessment tool (Higgins et al., 2011). All studies were assessed for risk of bias due to allocation of interventions (Selection bias), blinding of personnel (Performance bias), and blinding of outcome assessment (Detection bias). This was performed at the individual study level as opposed to the outcome level. This risk of bias was assessed by two Master level students (K. O’Neill, P. Gerber), with familiarity in executing challenge studies in veterinary medicine, but no familiarity with conducting field trials or formal training in biases of clinical trials in veterinary science. Disagreements in bias assessment were discussed and resolved by the students during the compilation of the final data set.

IV. **Summary measures**

The summary measure of the outcome for each trial was the mean difference between treatments (ADG vaccinated arm – ADG unvaccinated arm). As our hypothesis was that the vaccines would increase ADG, we expected that the mean difference would be positive i.e., greater than zero.

V. **Synthesis of results**

The approach to synthesis was to conduct three pairwise meta-analyses. The summary effect measure for this outcome was the mean difference in ADG. A fixed effects and a random effects model were calculated using the Meta package in R version 2.15.1 (R Development Core Team). The hypothesis was that the overall difference was equal to the null value (mean difference = 0). A subgroup analysis based on the PRRSV status was also conducted. The aim of the subgroup analysis was to assess if PRRSV status was a source of heterogeneity. Overall and within subgroup heterogeneity was assessed using the chi-square test. The null hypothesis was that heterogeneity was not present. All tests of heterogeneity
were reported at the subgroup and group level. First, subgroup heterogeneity was assessed. If the p-value for heterogeneity between the subgroup effects was significant (P<0.01), it was inferred that the subgroups were different. If the p-value for subgroup heterogeneity was greater than 0.01, the subgroup was not a source of heterogeneity. The subgroups were then collapsed, and heterogeneity was assessed across the entire population. We also reported the I² which describes the percentage of variation across studies due to heterogeneity rather than chance. I² is an intuitive and simple expression of the inconsistency of studies’ results. The data was also used to create a forest plots for each pairwise comparison. We did not assess publication bias, the impact of group versus individual level allocation, or other methodological sources of bias as predictors of the outcomes.

VI. Risk of bias across studies

The Cochrane risk bias assessment tool (Higgins et al., 2011) was further used for evaluation of a potential bias within the cumulative results. All studies were assessed for risk of bias due to incomplete outcome data (Attrition bias) and selective reporting (Reporting bias). The Other bias portion of the assessment tool was utilized to identify if any authors associated with the study were employed by the manufacturer of the vaccine utilized in the trial.

The reporting source for all the trials was recorded and tabulated as to whether or not it was a refereed source, or if it was a conference proceeding.

5. Results

1. Study selection
The database search yielded 985 articles overall, of which 161 were further examined for inclusion, based on the assessment of the title and abstract. Of these 161 articles, there were initially 41 duplicates. Additionally, articles in Czech (two articles), German (three articles), Hungarian, (one article), and Polish (one article) were excluded from consideration for the review. Within the initial 120 identified articles for inclusion through the database search, the publication types were refereed publications (n=69), non-refereed publications (n=39), and conference publications (n=12).

The search for supplemental information from vaccine manufacturers through the CVB resulted in three technical bulletins from Boehringer Ingelheim Animal Health Inc. (Sanford et al., 2007a; Sanford et al., 2007b; Sanford et al., 2008), one study from Pfizer Animal Health Inc. (Neuberger, 2011), and a veterinary product listing in the 12th Compendium of Veterinary Products (North American Compendiums Inc., 2010) for Merck Animal Health Inc. The technical bulletins from Boehringer Ingelheim Animal Health Inc. were excluded as they were duplicates of studies already identified through the database search. The study from Pfizer Animal Health Inc. was excluded as it did not describe a natural PCV2 exposure. The product listing for Merck Animal Health Inc. was a general statement of facts on the Circumvent® PCV product and was therefore excluded.

Screening of the titles of conference proceedings provided through AASV, the Allen D. Leman Swine Conference, IPVS, and the ISU Swine Disease Conference for Swine Practitioners proceedings yielded 174 articles of which 27 were duplicates of conference presentations or articles already included as part of the database search. The majority of relevant conference proceedings came from the IPVS congresses (92 studies), which outnumbered the combined amount of proceedings from the AASV conferences (43 studies),
the Allen D. Leman Swine Conferences (36 studies), and the ISU Swine Disease Conferences (3 studies). The majority of relevant conference proceedings, regardless of conference, were presented in 2010 (63 studies).

The combined total article count from all searches was 270 articles after initially discovered duplicates were eliminated (Fig. 1). The 233 articles which were excluded were further divided based on the selection criteria in the title, abstract, or text which rendered them not relevant to this study and which is demonstrated in Fig. 2.

II. Study characteristics

The results obtained for each vaccine are summarized in forest plots in Figs. 3, 4, and 5. Overall, there was a positive mean difference, regardless of vaccine product, demonstrating an increase in ADG with vaccination, although these mean differences were likely affected by the variance in sample sizes which was exacerbated by the different units of allocation. Additionally, one study (Nerem 2011) had an overly large variance (SD = ±430.732 g/day). This study only reported a Fisher’s exact test probability (p=0.04), and the results were back-calculated identically to other eligible studies which only reported p-values (see Outcome data).

III. Risk of bias within studies

Assessment of the risk of bias was performed for each study (Fig. 6). The two sources of bias which were most prevalent were the potential for Selection bias and Other bias, with approximately 50% of the included studies expressing each. The lowest risk of bias was observed with Attrition bias, with incomplete outcome data reporting only being encountered in one study.
IV. Synthesis of results

The results of the pairwise comparisons for Ingelvac® CircoFLEX, Circumvent® PCV, and Suvaxyn® PCV2/Fostera™ are summarized in Figs. 3, 4, and 5, respectively.

Of the 21 included studies, 13 were studies using the Ingelvac® CircoFLEX™ product. Overall, within the preferred random effects model, although the fixed effects model was reported as well, there was a mean difference of 27.84 g/day (95% CI, 20.06 – 35.63) between vaccinated (n=5,417) and unvaccinated (n=5,078) pigs (Fig. 3) and there was strong evidence of heterogeneity ($I^2 = 81.6\%$, $p<0.0001$). Furthermore, out of these 13 studies, eight involved PRRSV negative herds and the remaining five were conducted in PRRSV positive herds. The studies with a negative PRRSV status had a mean difference of 29.09 g/day (95% CI, 16.41 – 41.78) between vaccinated (n=3,235) and unvaccinated (n=2,959) pigs, while the studies with a positive PRRSV status had a mean difference of 25.84 g/day (95% CI, 18.26 – 33.43) between vaccinated (n=2,182) and unvaccinated (n=2,119) pigs. There was no difference between PRRSV positive and negative sub-groups ($p=0.66$), demonstrating little evidence of the PRRSV status influencing clinical heterogeneity, although when grouped by PRRSV status, the heterogeneity remained at the same level within the PRRSV negative trials ($I^2 = 87.3\%$, $p<0.0001$) but decreased in the PRRSV positive trials ($I^2 = 47.8\%$, $p = 0.1048$).

Of the remaining eight studies, six were studies using the Circumvent® PCV product. Due to lack of heterogeneity ($I^2 = 0\%$, $p=0.5884$) within these six studies, the fixed and random effects models were almost identical. Overall, there was a mean difference of 39.45 g/day (95% CI, 31.27 – 47.63) between vaccinated (n=587) and unvaccinated (n=544) pigs (Fig. 4). When sub-grouped by PRRSV status, there were two PRRSV negative studies with
a mean difference of 45.22 g/day (95% CI, 29.08 – 61.36) between vaccinated (n=419) and unvaccinated (n=415) pigs, and there were four PRRSV positive studies with a mean difference of 37.43 g/day (95% CI, 27.93 – 46.93) between vaccinated (n=168) and unvaccinated (n=129) pigs. Furthermore, there was no difference between sub-groups (p=0.41), suggesting strong evidence of no clinical heterogeneity.

The final two studies used Suvaxon® PCV2/Fostera™ PCV. Within the random effects model, there was a mean difference of 44.76 g/day (95% CI, -14.32 – 103.84) between vaccinated (n=385) and unvaccinated (n=375) pigs (Fig. 5). Furthermore, both studies were of PRRSV positive pigs and displayed strong evidence of heterogeneity ($I^2 = 90.5\%, p=0.0011$).

When tested for the overall effect, use of the Ingelvac® CircoFLEX™ and Circumvent® PCV vaccines resulted in significantly greater (p < 0.0001) daily weigh gains than their non-vaccinated counterparts (Fig. 3, 4). However, the average daily gain in pigs vaccinated with Suvaxon® PCV2/Fostera™ PCV was not different (p = 0.13) compared to pigs with no vaccination (Fig. 5) in our random effects model.

6. Discussion

1. Summary of evidence

The objective of this meta-analysis was to utilize published data on the three commercially licensed PCV2 vaccines that are currently available in the US, and create a comparison of vaccinated and non-vaccinated groups for each product with regards to ADG. While there are previous meta-analyses available, they focused on overall vaccine efficacy (Kristensen et al., 2011), the performance of Ingelvac® CircoFLEX™ when compared to
non-vaccinated animals (Holck et al., 2010; Coll et al., 2010), or the comparative efficacy of Ingelvac® CircoFLEX™ against Circumvent® PCV (Diaz et al., 2010). This is, to the authors’ knowledge, the first pairwise meta-analysis of all three of the commercially available PCV2 vaccines in the US relative to no vaccination.

Overall, two of the vaccines were demonstrated to contribute to significant increases in ADG. The Circumvent® PCV trials (n=6) demonstrated the most consistent and highest ADG increases (39.45 g/day) with the least heterogeneity ($I^2 = 0\%$) regardless of the herd PRRSV status, followed by the Ingelvac® CircoFLEX™ trials (27.84 g/day) for which we assessed data obtained from more individual studies (n=13) compared to the other two vaccines, but which also had a greater variation.

II. Limitations

The use of a random effects model was determined the most appropriate approach based on the assumption that statistical heterogeneity does not hold true (Higgins et al., 2011); however, this impacted the analysis of the Suvaxyn® PCV2/Fostera™ PCV trials. A higher weight was attributed to the smaller of the two qualified studies which potentially introduced a bias (Higgins et al., 2011). Furthermore, the estimate of the distribution was poor due to low numbers of the qualifying studies (Higgins et al., 2011), resulting in no detectable differences between treatment groups (vaccination; no vaccination) and a greatly exaggerated heterogeneity.

The use of conference proceedings in a systematic review can improve the pool of data for synthesis and minimize a potential bias from language limitations and associated exclusion of relevant studies (Sargeant et al., 2006), but can also complicate data synthesis. A
major limitation encountered from the addition of conference proceedings was the extremely variable methods of data presentation, resulting in conservative estimates of variance based on back calculation (Higgins et al., 2011) and a potential overall narrowing of the confidence interval due to inconsistent reporting of sample enrollment compared to mortalities as previously described (Booker et al., 2007).

Another concern with the inclusion of conference proceedings was the potential bias in inclusion. Of the 21 studies included in this meta-analysis, 10 were conference proceedings. Fifty percent (5/10) of these conference proceedings were deemed high risk for the Other bias during the risk of bias assessment due to authors being employed by the manufacturers of the vaccines, and could also not be associated with complementary refereed sources. Both of these factors can greatly impact the bias of the study, which is supported by a recent study suggesting a higher likelihood to report significant results through conference proceedings (Snedeker et al., 2010).

Within the Ingelvac® CircoFLEX™ trial portion of the Data synthesis, there were two studies which were determined to be outliers from the average mean difference (Maass 2010a and Ehlorsson 2010). These two outliers were small in size, contributing only 3.3% and 5.5% (Maass 2010a and Ehlorsson 2010, respectively) in the weighted random effects model. Interestingly, both of these studies were from PRRSV negative herds, and were isolated during the sub-group analysis, resulting in a lowering of the heterogeneity score for the PRRSV positive sub-group, although there was no significant difference between sub-groups. While these studies were likely contributing to the observed heterogeneity in this set of trials due to either within-study bias or increase of the study weight in the random effects model (Poole et al., 1999; Kjaergard et al., 2001; Higgins et al., 2011), it was decided to
leave these studies within the data set without sensitivity analysis. This was mainly done due to the varying sizes of the other studies (Higgins et al., 2011) and to maintain transparency within the reporting of this review (Liberati et al., 2009).

While there is a demonstrated difference in ADG between vaccination and no vaccination for two of the three products in this study, these data do not demonstrate comparative efficacy between these products. The pairwise analysis used does not compile a set of internally consistent estimates between the three models, nor does it maintain the randomization used in each study (Glenny et al., 2005; Dias et al., 2011). For comparative efficacy, a network meta-analysis (Dias et al., 2011) would be required.

**III. Conclusions**

All products reviewed in this pairwise meta-analysis demonstrated increases in ADG, though only Ingelvac® CircoFLEX™ and Circumvent® PCV demonstrated significant differences between vaccinated and non-vaccinated pigs. It is likely the small number of studies available for Suvaxyn® PCV2/Fostera™ PCV limited its applicability to the pairwise meta-analysis.

**7. References**


Bernabe, J., 2006. Vaccines of Mycoplasma and PMWS. [Vacunas de Mycoplasma y PMWS]. Albéitar. 95, 60.


Bischoff, R., Jedidia, S., Rocker, B., and Kamphake, M., 2009. Longitudinal study on the efficacy of Ingelvac CircoFLEX against Porcine Respiratory Disease Complex (PRDC)
[Langzeituntersuchung zur Wirksamkeit von Ingelvac CircoFLEX beim Porcinen Respiratorischen Krankheitskomplex (PRDC)]. Praktische Tierarzt. 90, 58-63.


Desrosiers, R., 2008. The impact of seroconversion following vaccination on the efficacy of PCV2 vaccination. Proc. 20th IPVS Congress 1, OR.01.024.


Ehlorsson, C.J., Blomqvist, G., Wallgren, P., 2010a. Vaccination against PCV2 in a herd that had been declared free from PMWS. Proc. 21st IPVS Congress 2, 423.


Estrada, E., 2011. The experience of Boehringer Ingelheim in controlling circovirus [La experiencia de Boehringer Ingelheim en el control de la circovirosis]. Suis. 84-87.


Glass, F., 2011. Vaccination against porcine circovirus type 2 is changing pig farming [Vakcinace proti PCV2 meni chovy prasat: nova era rizeni imunity u prasat]. Veterinářstvi. 61, 221-222.


Gonzalez, C., 2008. Vaccination provides superior control of PCV2 and PCVD. Proc. 20th IPVS Congress 1, OR.01.046.


Menjon, R., Bollo, J., Jimenez, M., and Lopez, J., V, 2011. Control of clinical PCV2 by vaccination: is the optimum economic return obtained in all cases? [Control de la clinica por PCV2 mediante vacunacion: se obtiene en todos los casos el retorno economico optimo?] Suis. 80, 76-79.


Miyashita, M., Takahashi, Y., Yamaguchi, T., Shimomura, Y., 2010a. Willingness to approach behaviour in weaned piglets following vaccination with two different PCV2 vaccines in a Japanese commercial farm. Proc. 21st IPVS Congress 1, 172.


Papatsiros, V., 2011. PRRSV and PCV-2 infections in Greek swine farms: clinical forms and vaccination programmes. Vet. Scan. 6, 73.


USDA, 2011. Veterinary Services Memorandum No. 800.50.


Young, M., Cunningham, G., Sanford, E., 2008. Performance of Ingelvac CircoFLEX® vaccinated pigs in a subclinical PCVAD herd. Proc. 20th IPVS Congress 1, OR.01.25.

Young, M., Cunningham, G., and Sanford, E., 2011a. The productivity of pigs immunized with the vaccine Ingelvac CircoFLEX in a herd with a subclinical circovirus infection. Svinovodstvo (Moskva). 61-62.


Table 1. The PICOS-derived search parameters and input for the Boolean search string “Population of interest” AND “Outcome” AND “Intervention”. All search terms were established with the aid of a research librarian to account for lemmatization, regional spelling differences, and each database’s thesaurus.

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<th>Population of Interest</th>
<th>Outcome</th>
<th>Intervention</th>
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<tbody>
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<td>Barrow</td>
<td>Acute Pulmonary Edema</td>
<td>Immunisation</td>
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<tr>
<td>Barrows</td>
<td>APE</td>
<td>Immunise</td>
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<tr>
<td>Boar</td>
<td>Circoviridae</td>
<td>Immunised</td>
</tr>
<tr>
<td>Boars</td>
<td>Circovirus</td>
<td>Immunity</td>
</tr>
<tr>
<td>Feeder</td>
<td>PCV</td>
<td>Immunization</td>
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<td>Finishing</td>
<td>PCV2</td>
<td>Immunize</td>
</tr>
<tr>
<td>Gilt</td>
<td>PCV-2</td>
<td>Immunized</td>
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<td>Gilts</td>
<td>PCVAD</td>
<td>Immunoprophylaxis</td>
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<td>PCVD</td>
<td>Intervention</td>
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<td>PMWS</td>
<td>Interventions</td>
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<td>Vaccinate</td>
</tr>
<tr>
<td>Piglet</td>
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</tr>
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</tr>
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<td>Sow</td>
<td>Porcine respiratory disease complex</td>
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<td>Sows</td>
<td>Postweaning multisystemic wasting disorder</td>
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<tr>
<td>Swine</td>
<td>PRDC</td>
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Table 2. Summary of the resulting studies from the systematic review included in the Data synthesis. PRRSV status was determined from evidence in the studies or through contact with the investigators.

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Study ID</th>
<th>Intervention</th>
<th>PRRSV Status</th>
<th>Unit of Treatment Allocation</th>
<th>Study Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Criado, 2012)</td>
<td>Criado 2012</td>
<td>Ingelvac® CircoFLEX™</td>
<td>Negative</td>
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<td>457</td>
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<tr>
<td>(Dunlop et al., 2012)</td>
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<td>Ingelvac® CircoFLEX™</td>
<td>Negative</td>
<td>Pig</td>
<td>2,322</td>
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<tr>
<td>(Ehlorsson et al., 2010b)</td>
<td>Ehlorsson 2012</td>
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<td>Negative</td>
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<td>294</td>
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<tr>
<td>(Maass et al., 2009)</td>
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<tr>
<td>(Maass et al., 2009)</td>
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<td>672</td>
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<tr>
<td>(Young et al., 2011b)</td>
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<td>Pen</td>
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<td>(Arnold et al., 2008)</td>
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<td>(Fachinger et al., 2008)</td>
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<td>(Jacela et al., 2011)</td>
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Fig. 1. Flow chart diagramming the Study selection process.
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<td>(Bruguera et al., 2006; Bernabe, 2006; Umiza et al., 2006; Neuberger, 2006; Thacker, 2006; Opriessnig et al., 2006b; Loula, 2007; Francisco et al., 2007; Meng et al., 2007; Opriessnig et al., 2007a; Wu et al., 2007a; Wu et al., 2007b; Dupont et al., 2008; Madec et al., 2008; Wu et al., 2008; Kerkaert, 2008; Opriessnig, 2008; Hesse et al., 2008; Opriessnig et al., 2008a; Opriessnig et al., 2008b; Burch, 2008c; Dupont et al., 2009; Wu et al., 2009; Main, 2009; Andraud et al., 2009a; Madson et al., 2009a; Opriessnig et al., 2009a; Madson et al., 2009b; Andraud et al., 2009b; Opriessnig et al., 2009b; Sinha et al., 2010; Kekarainen et al., 2010; Petznick, 2010; Pittman et al., 2010; Luppi et al., 2010; Roof et al., 2010; Opriessnig et al., 2010a; Opriessnig et al., 2010b; Opriessnig et al., 2010c; Opriessnig et al., 2010d; Fablet et al., 2011; Anonymous 2011; Papatsiós, 2011; Madison et al., 2011; Trible et al., 2011; Cino-Ozuna et al., 2011; Kristensen et al., 2011; Gerber et al., 2011; Lehe et al., 2011; Opriessnig et al., 2011a; Opriessnig et al., 2011b; Opriessnig et al., 2011c; Rodriguez-Gomez et al., 2012; Oh et al., 2012; Shen et al., 2012; Beach et al., 2012; Tshering et al., 2012; Trible et al., 2012a; Trible et al., 2012b)</td>
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</table>

<table>
<thead>
<tr>
<th>Did not report required measures</th>
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<tr>
<td>(Ellis et al., 2006; Charreyre et al., 2006; Jacela et al., 2007; Erlandson et al., 2007; Nerem, 2007; Paulsson et al., 2008; Hii et al., 2008; Janice et al., 2008; Waddilove, 2008; Beek, 2008a; Joisel et al., 2008a; Fort et al., 2008a; Joisel et al., 2008b; Beek, 2008b; Cline et al., 2008b; Pejsak et al., 2009; Bergstrom et al., 2009; Yeske et al., 2009; Reindl et al., 2010; Joisel et al., 2010; Pejsak et al., 2010; Erlandson et al., 2010; Schmidt et al., 2010; Santos et al., 2010; Richardson et al., 2010; Kwang, 2010; del Campo et al., 2010; Eggan et al., 2010b; Menjón et al., 2011; Fraile et al., 2011; Liber et al., 2011; Togashi et al., 2011; Kurmann et al., 2011; Pejsak et al., 2011; Lyoo et al., 2011; Murray, 2011; Blood et al., 2011; Wilson et al., 2011; Pejsak et al., 2012; Fraile et al., 2012; Dan et al., 2012; Lopez et al., 2012; Wang et al., 2012; Dmitrik et al., 2012; Payne et al., 2012)</td>
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<th>Did not report a trial with 1 of the 3 listed vaccines against no vaccine</th>
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<td>(Connor et al., 2007; Kolb et al., 2007; Diaz et al., 2007; Desrosiers et al., 2007a; de Grau et al., 2007a; de Grau et al., 2007b; Desrosiers et al., 2007b; Kane et al., 2008; Desrosiers, 2008; Young et al., 2008; Petersen et al., 2008; Gonzalez, 2008; King et al., 2008; Paphavasit et al., 2008; Umiza et al., 2008; Mette et al., 2008; Neumann et al., 2008; Eichmeyer et al., 2008; Lyoo et al., 2008; Zivlavska et al., 2008; Segalès et al., 2008; Haas et al., 2008; Liesner et al., 2008; Edler et al., 2008; Bretey et al., 2008; Orveillon et al., 2008; Ramirez et al., 2008; Burch, 2008a; Thacker et al., 2008a; Ritzmann et al., 2008a; Cline et al., 2008a; Burch, 2008b; Fort et al., 2008b; Thacker et al., 2008b; Ritzmann et al., 2008b; Paphavasit et al., 2009; Desrosiers et al., 2009; Neumann et al., 2009; Kane et al., 2009; Connor et al., 2009; Eichmeyer et al., 2009; Duflo et al., 2009; Verbeck et al., 2009; Mellencamp et al., 2009; Hesse et al., 2009; Baysinger et al., 2009; Holck et al., 2009; Diaz, 2009; Maass et al., 2009; Bretey et al., 2009; Kelly et al., 2009; Suprenant et al., 2009; Shelton et al., 2009b; Rossi et al., 2010; Desrosiers, 2010; Burch, 2010; Takahagi et al., 2010; Connor et al., 2010; Lyoo et al., 2010; Almond et al., 2010; Yong et al., 2010; Channarong et al., 2010; Venegas-Vargas et al., 2010; Pittman et al., 2010; De Backer et al., 2010; Fleury et al., 2010; Havn et al., 2010; Olanday et al., 2010; Duangwhae et al., 2010; Yao et al., 2010; Liu et al., 2010; Oh et al., 2010; Grimbeek, 2010; Sy et al., 2010; DiPietre et al., 2010; Fourcron et al., 2010; Karunyasiri et al., 2010; Lorenzo et al., 2010; Cano et al., 2010; de Grau, 2010; Brons et al., 2010; Biksi et al., 2010; Holck et al., 2010; Diaz et al., 2010; Coll et al., 2010; Miller et al., 2010a; Miyashita et al., 2010a; Suprenant et al., 2010a; Eggan et al., 2010a; Kim et al., 2010a; Palacios et al., 2010a; Ehlorsson et al., 2010a; Miyashita et al., 2010b; Suprenant et al., 2010b; Miller et al., 2010b; Kim et al., 2010b; Palacios et al., 2010b; Kim et al., 2010c; Kim et al., 2010d; O'Dea et al., 2011; Strugnell et al., 2011; Lee et al., 2011; Alberti et al., 2011; Ramis et al., 2011; Nieto et al., 2012; Shelton et al., 2012; Lising et al., 2012; Toledo et al., 2012; Yu, 2012)</td>
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<th>Did not report vaccine trial without a natural PCV2 exposure</th>
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**Fig. 2.** List of all articles excluded with reasons for exclusion.
**Fig. 3.** Random and fixed effects models of all included trials for Ingelvac® CircoFLEX™, sub-grouped by PRRSV status with a forest plot of the observed mean differences and 95% confidence intervals.

*Barn was used as the unit of treatment allocation.*

†*Pen was used as the unit of treatment allocation.*
Fig. 4. Random and fixed effects models of all included trials for Circumvent® PCV, sub-grouped by PRRSV status with a forest plot of the observed mean differences and 95% confidence intervals.

†Pen was used as the unit of treatment allocation.
Fig. 5. Random and fixed effects models of all included trials for Suvaxyn® PCV2/Foster™ PCV, sub-grouped by PRRSV status with a forest plot of the observed mean differences and 95% confidence intervals.
**Fig. 6.** Summary of the risk of bias assessment for all included studies based on the review authors’ determination using the Cochrane risk bias assessment tool. (● = low risk of bias; ◦ = high risk of bias; ◼ = unclear risk of bias).
CHAPTER 3 – STUDIES ON PCV2 VACCINATION OF 5-DAY-OLD PIGLETS


Kevin C. O’Neill, Huigang Shen, Michelle Hemann, Nathan M. Beach, Xiang-Jin Meng,
Patrick G. Halbur, Tanja Opriessnig

1. Abstract

Porcine circovirus type 2 (PCV2) vaccines have become widely used since they became available in 2006. It is not uncommon for producers to use PCV2 vaccines in pigs younger than what is approved by manufacturers. The objective of this study was to determine the efficacy of a chimeric and a subunit PCV2 vaccine administered at 5 or 21 days of age. Forty-eight PCV2-naïve piglets were randomly divided into six groups of eight pigs each. Vaccination was done at day 5 or day 21, followed by triple challenge with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV) at day 49. Vaccinated pigs seroconverted to PCV2 approximately 14 days post-vaccination and had a detectable neutralizing antibody response by 21 days post-vaccination regardless of age at vaccination. At day 49, the pigs vaccinated with the chimeric vaccine had significantly higher levels of neutralizing antibodies than the pigs vaccinated with the subunit vaccine. After challenge, vaccinated pigs had significantly decreased levels of PCV2 viremia
and a decreased prevalence and severity of microscopic lesions compared to the positive-control group, which had severe lymphoid lesions associated with abundant PCV2 antigen, compatible with PCV-associated disease. The results of this study indicate that, under the conditions of this study, vaccination of PCV2-naïve pigs at day 5 or day 21 resulted in development of a detectable humoral immune response and provided reduction or complete protection against PCV2 viremia and PCV2-associated lesions after triple challenge with PCV2, PPV, and PRRSV.

2. Introduction

Porcine circovirus (PCV) is a circular, single-stranded, non-enveloped DNA virus (46) that can be separated into two main types: PCV type 1 (PCV1) and PCV type 2 (PCV2). PCV1 is not associated with disease or lesions in pigs and is commonly considered nonpathogenic (47). PCV2 is linked with a variety of clinical disease manifestations collectively referred to as PCV-associated disease (PCVAD), including systemic disease or postweaning multisystemic wasting syndrome (PMWS) (16), respiratory disease (17), and enteric disease (20) in growing pigs.

PCV2 is prevalent worldwide, and most herds are seropositive (5, 6, 47). From 2005 to 2006, PCVAD became increasingly problematic in North America, leading to high production losses for producers (19). Aggressive vaccination programs initiated in 2006 have substantially decreased the prevalence and severity of PCVAD (14, 21).

U.S. pork producers now have several choices of approved commercial vaccines. Two of the commercial PCV2 vaccines commonly used in the United States are a subunit vaccine and one is a chimeric vaccine. One of the subunit vaccines (Ingelvac CircoFLEX;
Boehringer Ingelheim Vetmedica) is licensed for use in pigs at 3 weeks of age or older, provides protection beginning 2 weeks post-vaccination, and has at least a 17-week duration of immunity. The inactivated chimeric PCV2 vaccine (formerly Suvaxyn PCV2 from Fort Dodge Animal Health, Inc., and now reformulated as Fostera™ PCV from Pfizer Animal Health, Inc.) is also licensed for use in pigs 3 weeks of age or older. According to the manufacturer, this product provides protection against PCV2 challenge 3 weeks (two-dose application) or 6 weeks (one-dose application) post-vaccination for up to 4 months duration. This product was voluntarily removed from the market in May 2010 due to concerns regarding the inactivation process (13) and was reintroduced to the market in August 2011.

In the field, coinfections heavily influence the severity and outcome of PCVAD. Some of the most severe field case reports of PCVAD describe coinfection of pigs with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV) (7, 8). PPV has been shown to cause stillbirths and mummification in breeding herds but is generally considered nonpathogenic in growing pigs (22); however, when pigs are co-infected with PCV2 and PPV, this can lead to severe PCVAD in a portion of the pigs (1, 8, 22). PRRSV has become endemic and is known to cause abortions in the breeding herd and pneumonia in growing pigs (39). When found combined with PCV2 in the field or when pigs are experimentally co-infected with PRRSV and PCV2, disease and lesions are often quite severe (2, 18, 40).

A common concern when evaluating a vaccination program, besides the efficacy of the vaccine, is the appropriate timing of vaccination to provide maximal protection for the pig and convenience of use for the pork producer. Vaccines are commonly labeled for use at day 21 or older. Many pig farm managers prefer to vaccinate pigs at day 2 to day 5, which is
when they are handling piglets for other reasons. There are concerns, ongoing discussions, and debate over whether the pig has a sufficiently mature immune system at this age and if passively acquired antibodies interfere with vaccination. Therefore, if vaccination against pathogens such as PCV2 is proven to be effective in pigs less than 1 week of age, this ultimately could lead to substantial changes in vaccination protocols on many farms.

The objective of this study was to determine the efficacy of two commercial PCV2 vaccines, an inactivated chimeric vaccine and a subunit vaccine, at day 5 and day 21, in a triple challenge model with PCV2, PPV, and PRRSV. The triple challenge model was used to mimic field conditions where coinfections with PCV2, PPV, and PRRSV are commonly observed (7, 8, 35, 36).

3. Materials and methods

1. Animals and housing

Forty-eight conventional cross-bred pigs were derived from six sows from a breeding herd known to be free of PCV2, PRRSV, and PPV as determined by routine serology conducted monthly. At 4 days of age, while still on the dam, all pigs were ear tagged and randomly assigned to one of six treatment groups within each litter so that at least one pig from each sow was in a given treatment group. The pigs were weaned at approximately 14 days of age and transported to the research facility. Upon arrival at the Iowa State University Livestock Infectious Disease Isolation Facility, the pigs were separated into four rooms: one room for the negative-control group, one room for the positive-control group, one room for both groups receiving the inactivated chimeric vaccine, and one room for both groups receiving the subunit vaccine. Pigs were housed in pens on a concrete floor that was cleaned
once daily. Each room had separate ventilation systems and one nipple drinker. The vaccinated pigs were separated in two pens placed on opposite sides of the room based on timing of vaccination at day 5 or day 21. All pigs were fed an age-appropriate diet free of animal proteins (excluding whey) and antibiotics (Natures Made; Heartland Co-op, Cambridge, IA).

II. Experimental design

The study was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC 11-09-6831-S) and the Institutional Biosafety Committee (IBC 09-I-0030-A). The 48 pigs were randomly divided into groups of 8 pigs. The timeline of the experiment is summarized in Fig. 1. At day 5, 16 pigs were vaccinated with one of two PCV2 vaccines: an inactivated chimeric vaccine (chimeric-d5) or a viral subunit vaccine (subunit-d5). Similarly at day 21, 16 pigs were vaccinated with the inactivated chimeric vaccine (chimeric-d21) or a subunit vaccine (subunit-d21). Upon arrival to the research facility, blood was collected at weekly intervals until termination of the project at week 10. The blood samples were collected in serum separator tubes (Becton Dickinson vacutainer; 8.5 ml) and centrifuged at 2,000 \( \times \) g for 10 min at 4°C, and the serum was separated into two aliquots and stored at −80°C until testing. All pigs, except for the negative-control group, were inoculated with PPV, PRRSV, and PCV2b at day 49, and all pigs were euthanized for necropsy at day 70.
i. **Clinical evaluations**

Upon arrival at the research facility the pigs were individually examined and then monitored daily for clinical signs of disease, such as inappetence, lethargy, lameness, and respiratory disease.

ii. **Vaccination**

The inactivated chimeric vaccine used in this study was Suvaxyn PCV2 (serial number 1861229A; Fort Dodge Animal Health, Inc.). The subunit vaccine was Ingelvac CircoFLEX (serial number 309-136; Boehringer Ingelheim Vetmedica). Each of the pigs in the vaccinated groups received 2 ml of Suvaxyn PCV2 or 1 ml of Ingelvac CircoFLEX vaccine intramuscularly into the right neck via a 0.77-mm 22-gauge needle. Vaccination was done at day 5 or day 21.

iii. **PCV2b, PPV, and PRRSV inoculation**

All pigs, excluding the negative-control group, were inoculated at day 49 with PCV2b, PPV, and PRRSV.

iv. **PCV2 inoculation**

The PCV2 inoculum consisted of PCV2b isolate NC-16845 (32), which was propagated on PK-15 cells to a titer of $10^{4.5}$ 50% tissue culture infective doses (TCID$_{50}$) per ml. PCV2 inoculation was done by administering 1 ml of the inoculum intramuscularly into the right neck and slowly dripping 2 ml of the inoculum intranasally (1 ml per nostril) while the pig was held in the upright position.
v. **PPV inoculation**

The PPV inoculum consisted of a tissue homogenate containing strain NADL-8 at a titer of $10^{6.0}$ TCID$_{50}$ per ml (25). PPV inoculation was done by slowly dripping 1 ml of inoculum intranasally while the pig was held in the upright position.

vi. **PRRSV inoculation**

The PRRSV inoculum consisted of PRRSV isolate ATCC VR2385 (15). PRRSV was propagated on MARC-145 cells to the seventh passage at a titer of $10^{5.0}$ TCID$_{50}$ per ml. PRRSV inoculation was done by slowly dripping 2.5 ml of inoculum intranasally while the pig was held in the upright position.

**III. Serology**

i. **PCV2**

All pig sera, from day 21 to day 70, were tested for anti-PCV2 antibodies by a PCV2 capsid protein-based enzyme-linked immunosorbent assay (ELISA) as previously described (28). A sample-to-positive (S/P) ratio of greater than or equal to 0.2 was considered positive. A fluorescent focus neutralization (FFN) assay was performed on serum samples collected 21 days after vaccination for all vaccinated pigs and at the day of challenge (day 49) for all pigs for the detection of neutralizing antibodies, using a previously described method (37).

ii. **PPV**

The anti-PPV IgG antibodies were detected in serum from day 49 and day 70 via a hemagglutination inhibition (HI) assay, as previously described (26).
iii. **PRRSV**

All pig sera from day 49 and day 70 were tested for anti-PRRSV antibodies by ELISA (PRRS X3Ab test; IDEXX Laboratories Inc., Westbrook, MA) according to the manufacturer's instructions. An S/P ratio of 0.4 was used as the minimum positive cutoff value.

**IV. Quantitative real-time PCR assays**

i. **Total nucleic acid extraction**

All day 49, day 56, day 63, and day 70 serum samples were extracted using a total nucleic acid extraction kit (MagMAX viral isolation kit; Applied Biosystems, Foster City, CA) with the KingFisher Flex magnetic particle processor extraction system (Thermo Fisher Scientific, Waltham, MA).

ii. **PCV2**

PCV2 viremia was determined by the detection of the presence and amount of viral DNA in serum samples from all pigs on day 49, day 56, day 63, and day 70 via quantitative PCR using the same primers and probes as previously described (42). This was done in a 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA). A final 25-μl volume containing 2.5 μl of extracted DNA was processed under the following thermocycler conditions: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.
iii. **PPV**

Viremia for PPV was determined by detection of the presence and amount of PPV DNA in serum samples collected on day 49, day 56, day 63, and day 70 via quantitative real-time PCR as previously described (42). The final volume of the reaction mixture was 25 µl, which consisted of 12.5 µl of commercially available master mix (TaqMan Universal PCR master mix; PE Applied Biosystems), 2.5 µl of DNA from either sample extraction or standard, 1 µl (0.4 µM) of each primer, and 0.5 µl (0.2 µM) of the probe. The thermocycler conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

iv. **PRRSV**

Quantitative real-time reverse transcription-PCR (RT-PCR) for PRRSV viremia was performed on serum samples collected on day 56, day 63, and day 70 using the TaqMan NA and EU PRRSV reagents (Applied Biosystem) as previously described (42). PRRSV RNA presence and quantity were identified with real-time RT-PCR by utilizing TaqMan NA and EA PRRSV reagents (Applied Biosystems) with a final volume of 25 µl, containing 12 µl of 2× multiplex RT-PCR buffer, 2.5 µl of 10× PRRSV primer probe mix, 1.25 µl of 20× multiplex enzyme mix, 0.75 µl of nuclease-free water, and 8 µl of either PRRSV RNA from the previous extraction or standards. The thermocycler conditions were as follows: 10 min at 45°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 70 s at 60°C.

V. **Necropsy**

All pigs were humanely euthanized with an intravenous overdose of pentobarbital sodium (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI) at day 70. The total amount of
macroscopic lung lesions was estimated and scored (0 to 100% of the lung affected) as previously described (15). The sizes of lymph nodes (score range from 0 to 3: 0 [normal], 1 [two times the normal size], 2 [three times the normal size], and 3 [four times the normal size]) were estimated as described previously (29). Sections of lung, heart, liver, lymph nodes (tracheobronchial, superficial inguinal, external iliac, mediastinal, and mesenteric), spleen, kidney, ileum, colon, tonsil, and thymus were collected, placed into 10% neutral buffered formalin, and routinely processed for histological examination.

**VI. Histopathology and immunohistochemistry**

Microscopic examination of tissues was done by a veterinary pathologist who was blinded to the treatment groups. Lung sections were scored for presence and severity of interstitial pneumonia, with scores ranging from 0 to 6 (0 [normal]; 6 [severe diffuse]) (15). Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored on a scale of 0 (none) to 3 (severe). Lymphoid tissues, including lymph nodes, tonsil, and spleen, were evaluated for the presence of lymphoid depletion, with scores ranging from 0 (normal) to 3 (severe lymphoid depletion) and scores for histiocytic replacement of follicles ranging from 0 (normal) to 3 (severe) (34).

Immunohistochemistry (IHC) for PCV2 was performed on formalin-fixed, paraffin-embedded tissue sections by using a rabbit polyclonal antibody as previously described (45). Tissues evaluated included tonsil, spleen, lymph nodes (mesenteric, mediastinal, tracheobronchial, external inguinal, and subiliac), and thymus. PCV2 antigen scoring was performed in a blinded fashion, and scores ranged from 0 (no signal) to 3 (more than 50% of lymphoid follicles containing cells with PCV2 antigen staining) (34).
The overall PCV2-associated lesion scores were determined as previously described (34). A combined scoring system for each lymphoid tissue that ranged from 0 to 9 (lymphoid depletion score, 0 to 3; histiocytic replacement score, 0 to 3; PCV2 IHC score, 0 to 3) was used. The scores (lesions and PCV2 IHC) of the seven lymphoid tissues (lymph node pool × 5, spleen, and tonsil) were added together and divided by 7. The lymph node pool consisted of one section each of tracheobronchial, superficial inguinal, external iliac, mediastinal, and mesenteric lymph nodes. Pigs were grouped into four categories based on overall microscopic lymphoid lesion score: normal (score of 0), mild (score of 1 to 3), moderate (score of 4 to 6), and severe (score of 7 to 9). A pig was diagnosed with PCVAD if the mean lymphoid microscopic lesion severity score was severe (score of 7 to 9). The mean group overall lymphoid score was calculated and compared between groups.

VII. Statistical analysis

The data were statistically analyzed by performing a one-way analysis of variance (ANOVA) with JMP software version 9.0.0 (SAS Institute, Cary, NC). The significance level was $P < 0.05$, followed by pairwise testing using the Tukey-Kramer adjustment to identify the groups that were different. All real-time PCR data were log$_{10}$ transformed prior to analysis. The percentage of reduction of PCV2 viremia in vaccinated groups compared to the non-vaccinated positive-control group was calculated as follows: $100 - \left[ (100 \times \text{mean log}_{10} \text{genomic copies per ml of serum in vaccinated animals}) \big/ (\text{mean log}_{10} \text{genomic copies per ml of serum in positive-control animals}) \right]$. Nonrepeated measures, such as histopathology data, were assessed using a nonparametric Kruskal-Wallis ANOVA. If a nonparametric ANOVA
test was significant \( P < 0.05 \), then Wilcoxon tests were used to assess the differences of pairs of groups. Differences in incidence were evaluated by using Fisher's exact test.

4. Results

1. Clinical disease

After challenge, triple-challenged pigs in all groups developed mild to severe respiratory disease characterized by sneezing, increased respiratory rates, and clear nasal discharge. A portion of the triple-challenged pigs also became lethargic.

II. Seroconversion against PCV2, PPV, and PRRSV

i. PCV2

The negative-control pigs remained seronegative until termination of the study (Fig. 2 A). Seroconversion to PCV2 in the vaccinated groups was similar for the day 5 (Fig. 2B) and day 21 (Fig. 2C) groups. By 14 days post-vaccination, 2/8 subunit-d5, 3/8 subunit-d21, 7/8 chimeric-d5, and 8/8 chimeric-d21 animals had seroconverted; by 21 days after vaccination all vaccinated pigs except 2/8 subunit-d21 animals had seroconverted; by 28 days after vaccination all vaccinated pigs were seropositive for PCV2. There was a trend to lower levels of detectable anti-PCV2 IgG in pigs vaccinated with the subunit vaccine compared to those vaccinated with the chimeric vaccine, and this was independent of age of vaccination (Fig. 2B and C). The mean amounts of neutralizing antibody levels 21 days post-vaccination were similar in pigs vaccinated at day 5 (mean group log\(_{10}\) titers of 1.84 ± 0.16) (± standard error [SE]) and day 21 (1.56 ± 0.12); however, there was a significant difference when the data were analyzed by product (2.01 ± 0.14 for the chimeric vaccine and 1.39 ± 0.11 for the subunit vaccine)
As expected, when the data were evaluated by day of age rather than by days after vaccination, vaccination at day 5 resulted in significantly ($P < 0.05$) higher anti-PCV2 IgG levels from day 21 until day 42; however, there were no differences between the day 5- and day 21-vaccinated groups thereafter (Fig. 2A). At day 21, anti-PCV2 IgG was detected in 25% (2/8) of the subunit-d5 pigs and 87.5% (7/8) of the chimeric-d5 pigs. The prevalence of seropositive pigs was 100% at day 28 for the day 5-vaccinated pigs and 18.8% (3/16) for the day 21-vaccinated pigs. All pigs in these groups were seropositive for PCV2 by day 42. Regardless of timing of vaccination, the chimeric vaccine induced significantly ($P < 0.05$) higher levels of neutralizing antibodies at day 49 than the subunit vaccine, with mean group log$_{10}$ titers of 2.38 ± 0.18 for the chimeric vaccine compared to 1.82 ± 0.12 for the subunit vaccine. Positive-control pigs started to seroconvert by day 63 (62.5%; 5/8 pigs) and day 70 (75%; 6/8 pigs) as detected by ELISA.

**ii. PPV**

All groups were negative for anti-PPV antibodies on the day of challenge (day 49), and the non-challenged negative controls remained negative until day 70. All pigs challenged with PPV seroconverted by day 70; however, 2/8 positive-control pigs had noticeably lower titers (1:2,048) than all other pigs (1:4,096 to >16,384). Overall, the mean group PPV titers of the PPV-challenged animals were not different among treated groups (data not shown).

**iii. PRRSV**

All pigs in all groups were negative for anti-PRRSV IgG on the day of challenge (day 49), and the non-challenged negative controls remained negative until day 70. The majority of the pigs challenged with PRRSV seroconverted by day 70, with the exception of 2/8
positive-control pigs. Overall, the mean group anti-PRRSV IgG S/P ratios of the PRRSV-challenged pigs were not different among groups (data not shown).

**III. PCV2, PPV, and PRRSV viremia**

**i. PCV2**

All pigs were negative for PCV2 DNA at the day of challenge (day 49), and the negative-control pigs remained negative for PCV2 DNA in serum until termination of the study at day 70. The prevalence and the log$_{10}$ mean group amount of PCV2 DNA in the challenged groups are summarized in Table 1. All vaccinated groups had significantly ($P < 0.05$) smaller amounts of PCV2 DNA in serum than the positive-control group. When the data were divided based on age of vaccination, no evidence of an effect of age at vaccination on PCV2 viremia was seen. However, pigs vaccinated with the chimeric vaccine had significantly lower mean amounts of PCV2 genomic copies in serum samples on day 63 ($P = 0.021$) and day 70 ($P = 0.03$) than those vaccinated with the subunit vaccine. After challenge, PCV2 viremia was reduced by 75.4% to 100% in the vaccinated groups compared to the positive-control group.

**ii. PPV**

All pigs were negative for PPV DNA at the day of challenge (day 49), and the negative-control pigs remained negative until the termination of the study. The prevalence of PPV DNA positive pigs at day 56 was 100% for subunit-d5 and subunit-d21, and it was 88.9% (7/8) for the chimeric-d5, chimeric-d21, and the positive-control groups. The overall prevalence rate of PPV DNA-positive animals was 68.8% (33/48) by day 63 and 20.8% (10/48) by day 70, with no significant differences among groups.
iii. **PRRSV**

All pigs were negative for PRRSV RNA at the day of challenge (day 49), and the negative-control pigs remained PCR negative throughout the study. PRRSV RNA was detected in all PRRSV-challenged pigs on day 56, day 63, and day 70 without significant differences in the mean group PRRSV RNA levels among the challenged groups, regardless of vaccination status.

**IV. Gross lesions**

There were no visible gross lesions in the non-infected control pigs. A portion of the triple-challenged pigs, regardless of vaccination status, had moderate to severe mottled, tan-colored, consolidated areas of lung tissue involving up to 51% of the lung surface. A portion of the pigs had lymph nodes that were up to three times the normal size. There were no significant differences in gross lesions between challenged pigs.

**V. Microscopic lesions and presence of PCV2 antigen in tissues**

The majority of the pigs developed mild to severe interstitial pneumonia lesions characterized by thickening of alveolar septa by macrophages and lymphocytes and mild to severe type 2 pneumocyte hypertrophy and hyperplasia. The mean group interstitial pneumonia scores ranged from 3.0 ± 0.1 to 3.6 ± 0.4 in the triple-challenged groups and were significantly higher \((P < 0.05)\) than for the negative-control group \((0.8 ± 0.1)\). Lymphoid lesions, if present, were characterized by mild to severe lymphoid depletion and mild to severe histiocytic replacement of lymphoid follicles.

The prevalence rates of PCV2 antigen and overall lymphoid lesion scores for the different groups are summarized in Table 2. The majority of vaccinated pigs had no
remarkable lesions and were considered normal. Individual vaccinated pigs (7/32) had an overall lesion score of 1 or 2. In the positive-control group, 25% (2/8) of the pigs had microscopic lesions compatible with PCVAD associated with abundant amounts of PCV2 antigen and an overall lymphoid score of 9; 37.5% (3/8) of the pigs had moderate lymphoid lesions; the remaining 37.5% (3/8) of the pigs had no to mild lymphoid lesions.

5. Discussion

The main objective of this study was to determine the efficacy of PCV2 vaccination at an earlier age than recommended by the vaccine manufacturers. Several research groups have studied the efficacy of commercial PCV2 vaccines in pigs singularly infected with PCV2 (30, 31) or in pigs concurrently infected with multiple pathogens (33, 42). In all previous studies, vaccination was done according to the manufacturer's label instructions. To our knowledge, this is the first controlled experimental study to test the efficacy of commercial vaccines used at less than 3 weeks of age in a manner not approved by the manufacturer; however, this regimen mimics what is commonly now done in the field in the United States. Many producers prefer to vaccinate with a single-dose PCV2 product while piglets are undergoing castration, iron shots, tail docking, and teeth clipping between 2 and 5 days of age. However, there is concern that the immune system may not be mature enough to effectively respond to the vaccinations, potentially resulting in decreased vaccine efficacy and duration of immunity. To evaluate the benefits and shortcomings of early vaccination, this study entailed use of piglets blocked by litter and randomly assigned to early vaccination (day 5), regular vaccination (day 21), or no-vaccination (positive- and negative-control) groups.
After challenge, PCV2 viremia and associated lesions were similarly reduced in all vaccinated pigs regardless of timing of vaccination, indicating that day 5 pigs are capable of mounting a protective immune response. Vaccinated pigs were protected from development of PCV2-associated lesions independent of timing of vaccination, further indicating that both day 5 and day 21 vaccination protocols with either vaccine were effective. The pig immune system is unique in many ways that may be responsible for its ability to develop protective immunity from early vaccination. These factors include the full-length complementarity-determining region 3 (CDR3) of the heavy chain of immunoglobulin (4), limited genetic combinatorial pre-immune repertoire development (4), and the absence of true gene conversion sometimes seen in other species (44). The above-mentioned characteristics of the pig immune system combined with the results of this study demonstrate that the 5-day-old suckling pig is indeed capable of mounting a protective immune response against PCV2 challenge.

The current study was done in PCV2-naïve pigs; however, under field conditions most pigs that have suckled will be seropositive due to the ubiquitous nature of PCV2 and high levels of anti-PCV2 antibodies in colostrum. Interference with vaccination against swine influenza virus associated with the presence of passively acquired antibodies has been documented (3, 23, 27, 38); however, evidence of passive antibody interference with PCV2 vaccination has not been confirmed under experimental conditions (30). Furthermore, PCV2 vaccines have been highly effective in the field, and almost all pigs are seropositive to PCV2 at the time of PCV2 vaccination (9, 19, 21, 41). In experimental PCV2 challenge models, outcomes are often similar between vaccine treatment groups (11, 24), and conclusions often lack power. Passively acquired antibodies in many instances decrease PCV2 viremia and
prevent the development of clinical disease under controlled experimental conditions. PCV2 viremia and expression of clinical disease are often the main outcomes used for vaccine efficacy comparisons; however, when using animals with maternally derived anti-PCV2 antibodies, a much larger sample size may be required to demonstrate differences. Although the antibody-negative status of the pigs in the current study did not necessarily mimic what occurs with the majority of pigs in the field, studies performed in PCV2 antibody-free and PCV2 virus-free pigs are an important first step to increasing our understanding of potential advantages and disadvantages of early vaccination regimens.

To determine if there were differences in the efficacy of one vaccine over another, two different products were used side by side in this study. Several previous studies had been performed to determine the efficacy of PCV2 subunit vaccines and chimeric PCV2 vaccines (10, 12, 43). In these studies, vaccinated animals were shown to have strong antibody responses associated with decreased PCV2 viremia after challenge. Similarly, in our study the vaccinated animals, regardless of the type of PCV2 vaccine used, all developed a detectable antibody response and protective immunity as evidenced by significantly decreased PCV2 viremia and a decreased incidence and severity of lesions compared to the positive-control group. However, pigs vaccinated with the chimeric product had a stronger anti-PCV2 IgG response that was independent of age at vaccination and a lower prevalence of PCV2 viremic animals at day 63 and day 70 than pigs vaccinated with the subunit product. Moreover, and similar to a previous study using single-dose vaccination (42), vaccination with the chimeric product was associated with production of a stronger neutralizing antibody response than vaccination with the subunit vaccine.
In summary, under the conditions of this study, vaccination with chimeric or subunit PCV2 vaccines at 5 or 21 days of age induced a protective immune response in PCV2-naïve pigs as demonstrated by development of anti-PCV2 antibodies and reductions of PCV2 viremia and PCV2-associated lesions in a triple challenge model with PCV2, PPV, and PRRSV.

6. Acknowledgments

This study was funded by the National Pork Board Pork Checkoff Dollars. We thank Shayleen Schalk for assistance with the animal work.

7. References


Table 1. Prevalence and mean $\log_{10}$ PCV2 DNA in pigs challenged with PCV2 at age 49 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Prevalence (mean level ± SE)$^a$ of $\log_{10}$ PCV2 DNA on day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Subunit – d21</td>
<td>1/8</td>
</tr>
<tr>
<td></td>
<td>(0.7 ± 0.7)$^A$</td>
</tr>
<tr>
<td>Subunit – d5</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>(0.0)$^A$</td>
</tr>
<tr>
<td>Chimeric – d21</td>
<td>1/8</td>
</tr>
<tr>
<td></td>
<td>(0.6 ± 0.6)$^A$</td>
</tr>
<tr>
<td>Chimeric – d5</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>(0.0)$^A$</td>
</tr>
<tr>
<td>Positive Controls</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>(4.1 ± 0.6)$^B$</td>
</tr>
</tbody>
</table>

$^a$Different superscript capital letters (A, B, and C) within a column indicate significantly ($P < 0.05$) different amounts of group mean PCV2 DNA.
Table 2. Lymphoid depletion score and prevalence of PCV2 antigen in lymphoid tissues as determined by IHC.

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall lymphoid lesion score (mean ± SE)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Prevalence of PCV2 antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subunit – d21</td>
<td>0.11 ± 0.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1/8</td>
</tr>
<tr>
<td>Subunit – d5</td>
<td>0.38 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2/8</td>
</tr>
<tr>
<td>Chimeric – d21</td>
<td>0.09 ± 0.09&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0/8</td>
</tr>
<tr>
<td>Chimeric – d5</td>
<td>0.30 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2/8</td>
</tr>
<tr>
<td>Negative Controls</td>
<td>0.0 ± 0.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0/8</td>
</tr>
<tr>
<td>Positive Controls</td>
<td>4.59 ± 1.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7/8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant differences among groups are indicated by different superscript capital letters (A and B).
Fig. 1. Experimental design. All serum samples collected were tested for the presence of PCV2 antibody. Samples from day of age 49, 56, 63, and 70 were tested for the presence and amount of PCV2 DNA and PPV DNA. Samples from day 56, day 63, and day 70 were tested for the presence and amount of PRRSV RNA. Samples from day 49 and day 70 were tested serologically for PRRSV and PPV.
Fig. 2. (A) Mean group PCV2 ELISA S/P ratios (± SE) on serum collected from piglets vaccinated at day of age 5 (d5) or 21 (d21) or non-vaccinated and challenged with PCV2, PPV, and PRRSV at day 49, which corresponds to 44 days after vaccination for day 5 piglets and 28 days after vaccination for day 21 piglets. An S/P ratio of 0.2 or greater was considered seropositive. Significant differences among groups on a certain day are indicated.
by different letters (A, B, and C). (B) Comparison of subunit-d5 and chimeric-d5 pigs at different days post-vaccination. Significant differences among groups on a certain day are indicated by an asterisk. (C) Comparison of subunit-d21 and chimeric-d21 pigs at different days post-vaccination. Significant differences among groups on a certain day are indicated by an asterisk.
CHAPTER 4 – VACCINATION OF SOWS REDUCES THE PREVALENCE OF PCV2 VIREMIA IN THEIR PIGLETS UNDER FIELD CONDITIONS

A paper published in the Veterinary Record [2012, October 27; 171 (17), 425-431]

Kevin C. O’Neill, Michelle Hemann, Luis G. Giménez-Lirola, Patrick G. Halbur, Tanja Opriessnig

1. Summary

The objectives of this study were to further understand vertical transmission of porcine circovirus type 2 (PCV-2) and the effect of dam vaccination on PCV-2 viraemia in newborn piglets. Randomly selected sows from each of two breeding herds were designated as non-vaccinated or vaccinated groups. A commercial inactivated PCV-2 vaccine was administered at weaning and 18 days later to half of the sows on each farm. At parturition, colostrum was collected from 70 dams on each farm and post- (Farm 1) or pre-suckle blood (Farm 2) was collected from five randomly selected piglets per litter. Colostrum samples had an anti-PCV-2 antibody prevalence of 98.5% (135/137) with significantly (p=0.0039) higher concentrations in vaccinated dams. Among piglets, 43.9% (301/685) were seropositive for PCV-2 and 11.7% (80/686) were PCV-2 DNA positive. All of the PCV-2 DNA positive samples were further characterized and 28 were PCV-2a, 28 PCV-2b, and 5 mixed PCV-2a and PCV-2b infection. The prevalence of PCV-2 DNA in piglets was lower (0.7% to 22.8%) compared to previous studies (44.8% to 90%) indicating a change in PCV-2 ecology likely
due to wide use of vaccination. Under the study conditions, dam vaccination reduced PCV-2 viraemia in the offspring with colostrum access.

2. Introduction

Porcine circovirus (PCV) type 2 (PCV-2) is a non-enveloped, single-stranded circular DNA virus (Tischer and others 1982) and along with PCV type 1 (PCV1) belongs to the family Circoviridae. PCV-2 is highly prevalent in the global swine population (Tischer and others 1986, Dulac and Afshar 1989, Edwards and Sands 1994, Segalés and others 2008) and can be divided into several genotypes of which PCV-2b is the predominant genotype (Cheung and others 2007, Gagnon and others 2007, Olvera and others 2007, Dupont and others 2008, Segalés and others 2008).

PCV-2 is linked to several clinical disease manifestations referred to as PCV-associated diseases (PCVAD) (Opriessnig and others 2007). Clinical PCVAD may manifest as postweaning multisystemic wasting syndrome (PMWS) (Harding and Clark 1997), porcine respiratory disease (Harms and others 2002), enteric disease (Kim and others 2004), reproductive failure (West and others 1999) and less often as porcine dermatitis and nephropathy syndrome (PDNS) (Choi and Chae 2001). The ubiquitous nature of PCV-2 can be attributed to its many routes of transmission: oronasal (Shibata and others 2003), fecal (Shibata and others 2003), seminal (Larochelle and others 2000), trans-placental (Park and others 2005), and colostral (Shibata and others 2006). Vaccination of growing pigs against PCV-2 has been shown to be highly effective in decreasing the prevalence and severity of PCVAD (Kixmöller and others 2008, Gillespie and others 2009).
Recently, much attention has been given to PCV-2 prevention in growing pigs through vaccination of the breeding herd. It has been shown that PCV-2 infection of dams can result in viraemia and in utero infection of the piglets (West and others 1999, O'Connor and others 2001, Ladekjær-Mikkelsen and others 2001). Interestingly, a substantial number of piglets are born viraemic and appear to be healthy (Shen and others 2010a). In a recent field investigation in the USA, the frequency of vertical PCV-2 transmission in five commercial breeding herds demonstrated that the overall PCV-2 DNA prevalence was 69.5% (89/128) in sows and 44.8% (226/504) in neonates (Shen and others 2010a). Another research group determined that prevalence rates of PCV-2 viraemic newborn pigs on three farms ranged from 58% to 90% (Dvorak and others 2010).

The objectives of this study were to determine if the previous results of high levels of PCV-2 viraemia in dams and piglets occurs in breeding facilities in Iowa in 2011 and if dam vaccination has an effect on vertical PCV-2 transmission rates.

3. Materials and methods

1. Farms

Farm 1 was part of a multi-site, breed-to-wean facility that housed approximately 2,400 breeding age females. Dams were inseminated via artificial insemination (AI) using semen from a boar stud located in Iowa. Farm 2 was an individual breeding herd with approximately 2,700 breeding age females managed in similar fashion as Farm 1. All gilts and sows on both farms received a pre-farrow Escherichia coli vaccine. All breeding stock was routinely vaccinated against porcine parvovirus, leptospirosis, and Erysipelothrix rhusiopathiae at weaning, and dams on Farm 2 were also vaccinated against swine influenza.
virus. Both herds, although serologically positive for porcine reproductive and respiratory syndrome virus (PRRSV), were considered PRRSV stable, and produced PRRSV RNA negative piglets. Incoming gilts or resident breeding animals were not vaccinated against PCV-2. PCV-2 vaccination of breeding stock at weaning age prior to arrival in the breeding herds cannot be ruled out.

II. Experimental design, vaccination, sample collection and reproductive parameters

All study procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 11-09-6831-S). Calculation of the necessary sample size was derived from the assumption of a 50% prevalence of PCV-2 in breeding females similar to a previously described study (Shen and others 2010a). It was determined that on each farm, 35 dams in each treatment group were needed to detect a difference in effect. Therefore, on each of the two farms approximately 100 sows with concurrent oestrus cycles were blocked by parity (1-10) to allow for an equal parity representation, and divided into two groups of approximately 50 vaccinated and 50 non-vaccinated dams. Although samples were collected from only 35 sows, initially 50 sows were assigned to each group to assure that 35 sows would be farrowing at the time the research team visited the farm to collect samples. This allowed for any losses due to failure to conceive or missed or unsupervised farrowings. As soon as samples from 35 litters were collected, collection was immediately stopped and samples and data from the remaining litters were not collected.

The vaccinated group was vaccinated with Suvaxyn PCV (Fort Dodge Animal Health Inc., serial number: 1861229A) at weaning. The vaccine was administered in a 2 ml dose intramuscularly in the right neck. A second booster vaccination was administered 18 days
later using 1 ml intramuscularly at the same site. All sows were routinely inseminated during their first oestrus cycle after weaning.

Among the selected sow parity blocks, for each treatment group 35 sows were arbitrarily selected at the time of farrowing. At parturition, colostrum was collected from the dams and blood was collected from five arbitrarily selected healthy appearing piglets from each litter prior to colostrum uptake. Based on evaluation of swine total IgG optical density (OD) values in piglet sera, it was concluded that sample collection occurred after colostrum on Farm 1 and prior to colostrum uptake on Farm 2. The piglets on Farm 1 were selected by farm workers and brought into a different room to the research team members who were responsible for blood collection. Blood was collected from the umbilical cord or the jugular vein. To avoid cross contamination during sample collection, gloves were changed between animals and tubes were opened, closed and stored by people not handling the animals. On Farm 2, piglet selection and samples collection was solely done by research team members. Blood was collected only from the jugular vein. The final sample numbers obtained are summarized in Table 1. All samples were tested for the presence of anti-PCV-2 IgG via serology, and presence, quantity, and subtype of PCV-2 DNA through real-time PCR assays. At farrowing, litter characteristics were noted for all dams included in this study.

III. Serology

All colostrum and serum samples were tested for anti-PCV-2 antibodies using a previously described ORF2-based enzyme linked immunosorbent assay (ELISA) (Nawagitgul and others 2002). A sample with a sample-to-positive (S/P) ratio equal or greater than 0.2 was considered positive. Selected samples (n=44) obtained from Farm 1
were also tested with the SERELISA PCV2 Ab Mono Blocking ELISA kit (Synbiotics; Pfizer, Inc), according to manufacturers’ instructions.

All piglet serum samples were also tested by an in-house swine total IgG ELISA. Briefly, microtiter plates (Nunc; Thermo Fisher Scientific Inc) were coated with 0.5 μg/ml of a goat anti-swine IgG (Fc) antibody (Fitzgerald) in phosphate-buffered saline (PBS) and were incubated overnight at room temperature. After three washes with PBS containing 0.05% Tween 20, the plates were blocked with 1% bovine serum albumin (Jackson ImmunoResearch, Inc) for 2 h at room temperature. The serum samples were diluted 1:100 with PBS containing bovine serum albumin (Jackson ImmunoResearch, Inc), were placed on the plates, and were incubated for 45 min at 37°C. After a washing step, a 1:40,000 dilution of peroxidase-conjugated 125 goat anti-swine IgG (Jackson ImmunoResearch, Inc) was added to each well and incubated at 37°C for 30 min. After another washing step, tetramethylbenzidine-hydrogen peroxide (TMB) chromogenic substrate (KPL) was added to each well. After 15 min at room temperature the reaction was terminated by adding 50 μl of 2.5 M sulfuric acid to each well and the absorbance at 450 nm was measured using a spectrophotometer. As assay controls, piglets sera with known suckle or pre-suckle status were included. The piglet mean OD readings were compared across farms.

IV. Detection of PCV-2 DNA

i. DNA extraction.

DNA from all serum and colostrum samples was obtained by using a commercially available extraction kit (QIAamp DNA Blood Kit; Qiagen) according to the manufacturers’ specifications.
**ii. General PCV-2 real-time PCR.**

PCV-2 DNA was detected using previously described primers and a probe targeting ORF1 of PCV-2 (Shen and others 2010b) in a 7500 Fast Real-Time PCR system (ABI). The reaction consisted of a total volume of 25 µl containing 2.5 µl of extracted DNA and processed in the following cycles: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, and 40 cycles of 1 min at 60°C. A sample with a cycle threshold (C_T) value greater than 40 was considered negative.

**iii. PCV-2a/b multi-plex PCR.**

Differentiation between PCV-2a and PCV-2b was achieved by using a previously described multiplex real-time PCR assay targeting signature motif located in ORF2 of PCV-2 (Opriessnig and others 2010) with a total reaction volume of 25µl consisting of 12.5 µl of commercially available master mix (TaqMan Universal PCR master mix), 5 µl of DNA, 0.4 µM of each primer, and 0.2 µM of each probe. The cycling conditions were as follows: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, and 40 cycles of 1 min at 60°C. A sample with a C_T value greater than 40 was considered negative.

**V. Statistical analysis**

Quantitative real-time PCR results were log_{10} transformed before analysis. Data analysis was performed using JMP software version 9.0.0 and SAS software version 9.2 (SAS Institute) and a p-value less than 0.05 was considered to indicate statistical significance. For ELISA S/P ratios, a linear mixed model was used with “farm”, “parity” and “farm by vaccination status interaction” as fixed effects. For binary outcomes (PCV-2 antibody prevalence or DNA prevalence), Cochran-Mantel-Haenszel statistics were used to
assess association between “vaccination status” and “PCV-2 prevalence rates” stratified by parity and farm. Serological results for each farm were analyzed using pairwise t-tests. Differences in prevalence between groups were determined by Fisher’s exact test.

4. Results

1. Litter characteristics

Among the selected litters in Farm 1, the average number of liveborn pigs (se) was 12.2 (0.3), the average number of stillborn pigs was 1.2 (0.2), and the average number of mummified fetuses was 0.4 (0.2). Among the selected litters in Farm 2, the average number of liveborn pigs was 11.8 (0.4), the average number of stillborn pigs was 0.8 (0.2), and the average number of mummified fetuses was 0.2 (0.1). There were no differences (p>0.05) between vaccinated and non-vaccinated dams (Table 2).

II. Total IgG levels in piglet sera

The anti-IgG OD values from Farm 1 piglets were similar to samples obtained from piglets collected after suckling whereas those from Farm 2 piglets were similar to samples obtained from piglets prior to suckling (data not shown). Farm 1 piglets had significantly (p<0.0001) higher mean OD values (1.32±0.02) compared to Farm 2 piglets (0.63±0.01).

III. Prevalence of anti-PCV-2 IgG and mean group ELISA S/P ratios

Overall, all vaccinated dams (70/70) and 97% of the non-vaccinated dams (65/67) had detectable anti-PCV-2 IgG in colostrum. Vaccinated dams had significantly (p=0.0039) higher group mean S/P values compared to non-vaccinated dams (1.75±0.08 versus 1.41±0.07). There were no significant differences in prevalence rates of PCV-2-antibody
positive dams. The results for vaccinated and non-vaccinated dams by farm are summarized in Table 3.

Among piglets across both farms, 44.5% (153/344) of those derived from vaccinated dams and 43.4% (148/341) of the piglets derived from non-vaccinated dams had detectable anti-PCV-2 IgG in serum. The results for piglets derived from vaccinated and non-vaccinated dams separated by farm are summarized in Table 3. Due to the high numbers of PCV-2 seropositive piglets (88.1%; 297/337) on Farm 1, a portion of the seropositive samples (n=44) was tested with a second commercial available ELISA and similar results were obtained (data not shown). Farm 1 piglets obtained from vaccinated dams had significantly (p<0.001) higher anti-PCV-2 IgG than piglets derived from non-vaccinated dams (Table 3) which was independent of total IgG levels (p=0.203). On Farm 2, only 1.1% (4/348) of the piglets were seropositive. Due to the low numbers of seropositive pigs, differences in mean group S/P ratios between piglets from vaccinated and non-vaccinated sows were not observed (Table 3).

The litter distribution of PCV-2 seropositive pigs separated by farm is summarized in Table 4. There were no differences in prevalence of seropositive pigs between litters from vaccinated or non-vaccinated dams within farms.

IV. Prevalence of PCV-2 DNA

Overall, PCV-2 DNA was identified in 2.9% (2/67) of colostrum samples from non-vaccinated dams but was not detected in colostrum from any of the 70 vaccinated dams. The prevalence of PCV-2 DNA separated by treatment status and farm is summarized in Table 3. PCV-2 DNA was identified in 5.9% of the colostrum samples obtained from non-vaccinated
Farm 1 sows (2/34). PCV-2 DNA was not detected in colostrum from any of the vaccinated dams in Farm 1 or in colostrum from any of the vaccinated or non-vaccinated dams in Farm 2 (Table 3).

The combined Farm 1 and Farm 2 results indicated that PCV-2 DNA was detected in 14.9% (51/342) of the serum samples obtained from piglets derived from non-vaccinated dams and in 8.4% (29/344) of the samples obtained from piglets derived from vaccinated dams. Among PCV-2 positive samples, 28 were identified as PCV-2a, 28 were PCV-2b, five had a mixed PCV-2a and PCV-2b infection and the subtype was not determinable in 19 PCR positive piglets. On farm 1, piglets derived from vaccinated dams had a significantly (p<0.001) lower prevalence of PCV-2 DNA positive pigs compared to piglets obtained from non-vaccinated dams (Table 3). The litter distribution of PCV-2 DNA positive pigs separated by farm is summarized in Table 4. Overall, the prevalence of litters containing at least 1 to 3 PCV-2 DNA positive pigs was significantly (p=0.036) higher for litters from non-vaccinated sows (41.3%; 29/70) compared to litters obtained from vaccinated sows (28.6%; 20/70). On the individual farm level, for Farm 1 the prevalence of litters without PCV-2 DNA positive pigs was higher (p<0.01) if the pigs were born to vaccinated dams. Conversely, the prevalence of litters containing 2-3 PCV-2 DNA positive pigs was lower (p<0.05) if the pigs were born to vaccinated dams.

5. Discussion

This study aimed to confirm the previously determined high rates of vertical PCV-2 transmission and to evaluate potential benefits of PCV-2 vaccination of breeding herds on vertical PCV-2 transmission. To address these aims, two independent farrowing facilities that
were not currently utilizing PCV-2 vaccination in their breeding stock were identified and a portion of the dams were vaccinated with a commercially available inactivated vaccine.

Due to results from a previous study where we found comparable incidence of PCV-2 detection in serum (47.2%, 59/125) and colostrum samples (40.8%, 51/125) (Shen and others 2010a), to simplify the sample collection procedure in this study, only colostrum was collected and tested to determine the dam PCV-2 status. In this study, PCV-2 DNA was detected in 1.5% (2/137) of the colostrum samples which is lower compared to previous findings perhaps indicating a low grade PCV-2 infection in the selected farms. Nevertheless, the seroprevalence of PCV-2 antibody in colostrum samples was high (98.5%; 135/137) as expected. Interestingly, vaccinated dams had significantly (p=0.0039) higher group mean S/P values compared to non-vaccinated dams.

In this study the overall seroprevalence of PCV-2 antibody in newborn piglets was determined to be 43.9%. After initial analysis, the number of seropositive piglets (88.1%; 297/337) on Farm 1 was not consistent with previous findings on pre-suckle serum sample (Shen and others 2010a, Gerber and others 2011). Recent PCV-2 infection of the majority of the dams could have resulted in a high horizontal PCV-2 transmission rate after 70 days of gestation. However, considering the overall low prevalence rates of PCV-2 viraemic piglets (22.8%; 77/338) and of PCV-2 DNA positive colostrum samples (2.9%; 2/69), this seemed unlikely. Alternatively, the PCV-2 in-house ELISA could have generated false positive results. To rule this out, a subset of seropositive samples were tested with a different commercially available competitive ELISA and similar results were obtained. The method of blood collection, umbilical cord collection versus jugular vein collection could also have affected the outcome possibly due to contamination of the umbilical cord by maternal blood.
Finally, colostrum access of the piglets on Farm 1 was confirmed by detecting high total IgG OD levels in sera. The piglets were selected by farm workers and lack of colostrum uptake was not verified by research workers. It has been determined that colostral-derived antibody titres in neonatal pigs can be detected within 2 hours and may peak 9-24 hours after colostrum uptake (Lai and others 1986, Vandeputte and others 2001). On Farm 2, piglet selection and sample collection was done only by the research team members and therefore it was known that colostrum uptake did not occur in Farm 2 pigs. To account for the difference in colostrum access between the two farms, the piglet data were analyzed separately for each farm. PCV-2 vaccination had a significant (p<0.05) effect on piglet anti-PCV-2 IgG S/P ratios in Farm 1 with piglets born to vaccinated dams having higher group mean S/P ratios compared to piglets born to non-vaccinated dams.

Overall, the prevalence of PCV-2-DNA-positive piglets was 11.7%. In post-suckle serum samples obtained from Farm 1, there was significantly (p<0.001) lower prevalence of viraemic piglets from vaccinated dams (15.4%; 26/169), compared to piglets born to non-vaccinated dams. The same effect was also seen at the litter level [48.6%, (17/35) litters from vaccinated dams versus 82.9% (29/35) from non-vaccinated dams]. Interestingly, the detection rates of PCV-2a and PCV-2b were similar (28 samples each; 2/28 PCV2a positive samples were identified on Farm 2) which is in contrast to previous results which indicated a much higher rate of PCV-2b infection in piglet sera (69.5% versus 15.6%) based on analysis of 499 pre-suckle piglet serum samples (Shen and others 2010a). Contrary to previous studies, the current study utilized PCV-2 vaccination in a portion of the dams which may have interfered with PCV-2 subtype specific viral replication. It is well established that PCV-2 infection during early to mid-term gestation can cause sporadic reproductive failure (West
and others 1999, Johnson and others 2002, Sanchez, Jr. and others 2004, Mikami and others 2005). However, the impact of PCV-2 circulation in a breeding herd may be more important in the neonatal and growing pig than it is in the dam.

Higher incidences of PCVAD are often encountered after maternal antibodies begin to wane (Opriessnig and others 2004) when sub-clinically infected piglets are being comingled with naïve, growing animals. While the parameters of this study did not allow for observation of mortality and morbidity of the pigs throughout the growing period, the prevalence of viraemic piglets from vaccinated dams was significantly lower than in pigs born to non-vaccinated dams. In the field, this could lead to less and lower dose exposure to other pigs in the barn.

Although PCV-2 vaccination in growing pigs is now widespread and has resulted in decreased incidence of PCVAD (Kixmoller and others 2008, Gillespie and others 2009), indications are that they have not been entirely effective. There are several reports of PCV-2-associated severe, acute pneumonia with pulmonary edema in Kansas and Nebraska (Cino-Ozuna and others 2011). The emergence of a recombinant virus in Canada in late 2009 (Gagnon and others 2010) has also raised concerns about vaccine safety. While vaccination of sows has been shown ineffective in completely preventing intra-uterine infection from either PCV-2 spiked semen (Madson and others 2009a) or experimental challenge (Madson and others 2009b) in dams housed under experimental conditions, the results of this study corroborates previous findings that sow vaccination reduces the number of viraemic piglets by possibly hindering intrauterine infection (Shen and others 2010a) or decreasing infection via colostrum shortly after birth.
This study further adds to the knowledge on PCV-2 infection in breeding herds and the role of dam vaccination on PCV-2 prevalence in neonates. The overall prevalence of PCV-2 DNA in two Iowa breeding herds in 2011 was lower than reported in previous years. Under the study conditions, vaccination of the dams with a commercially available inactivated vaccine increased (p=0.0039) colostral anti-PCV-2 IgG. In addition, on post-suckle sera (Farm 1) vaccination reduced (p<0.001) the overall numbers of PCV-2 viraemic piglets and decreased (p=0.036) the prevalence of litters with PCV-2 viraemic pigs.

6. Acknowledgements

This study was funded by the National Pork Board Check Off Dollars. The authors thank Cody Branstad and Shayleen Schalk for assistance with the animal work, Kathy Lin for assistance with the laboratory testing, and Dr. Chong Wang for assistance with the statistical analysis.

7. References


**Table 1**: Number of samples of colostrum and serum obtained from PCV-2-vaccinated and non-vaccinated sows and corresponding piglets on two farms in Iowa, USA. Differences in sow numbers and litters are due to inability to collect colostrum from all of the dams.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Vaccination</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td>35</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>34</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Piglet serum</td>
<td>Yes</td>
<td>169</td>
<td>175</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>169</td>
<td>173</td>
<td>342</td>
</tr>
</tbody>
</table>
**Table 2:** Litter characteristics of the selected sows used in this study. Data presented as group mean (se).

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Farm</th>
<th>Liveborn</th>
<th>Stillborn</th>
<th>Mummified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>1</td>
<td>12.0 (0.5)</td>
<td>0.9 (0.2)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.3 (0.5)</td>
<td>0.9 (0.2)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>11.5 (0.6)</td>
<td>0.7 (0.4)</td>
<td>0.1 (0.0)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.3 (0.4)</td>
<td>1.4 (0.3)</td>
<td>0.3 (0.1)</td>
</tr>
</tbody>
</table>
Table 3: Prevalence of anti-PCV-2 antibodies, mean group ELISA sample-to-positive (S/P) ratios, and overall prevalence of PCV-2 DNA and prevalence of PCV-2 subtypes (PCV-2a, PCV-2b, or both).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Farm</th>
<th>Vaccination</th>
<th>Anti-PCV-2 IgG</th>
<th>PCV-2 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prevalence</td>
<td>Mean S/P ratios (se)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>100% (35/35)</td>
<td>1.32 (0.07)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>94.2% (32/34)</td>
<td>1.15 (0.09)</td>
</tr>
<tr>
<td>Dam colostrum</td>
<td>2</td>
<td>Yes</td>
<td>100% (35/35)</td>
<td>2.19 (0.10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>100% (33/33)</td>
<td>1.69 (0.08)</td>
</tr>
<tr>
<td>Piglet serum</td>
<td>1</td>
<td>Yes</td>
<td>89.9% (152/169)</td>
<td>1.42 (0.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>86.3% (145/168*)</td>
<td>1.07 (0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>0.6% (1/175)</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>1.7% (3/173)</td>
<td>0.03 (0.01)</td>
</tr>
</tbody>
</table>

*Insufficient quantity

A, B Indicates significant differences (p<0.001) in mean group S/P ratios for dams within a farm

I, II Indicates significant differences (p<0.001) in mean group S/P ratios for piglets with a farm

a, b Indicates significant differences (p<0.001) in PCV-2 DNA prevalence for piglets within a farm
Table 4: Prevalence of litters with 0, 1-2, or 3-4 or 5 anti-PCV-2 antibody positive pigs and prevalence of litters with 0, 1 or 2-3 PCV-2 DNA positive pigs from PCV-2 vaccinated or non-vaccinated dams at each of two farms.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Vaccination</th>
<th>Number of positive pigs per litter</th>
<th>Anti-PCV-2 IgG</th>
<th>PCV-2 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1-2</td>
<td>3-4</td>
</tr>
<tr>
<td>1</td>
<td>Yes</td>
<td>0% (0/35)</td>
<td>8.6% (3/35)</td>
<td>17.1% (6/35)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0% (0/35)</td>
<td>8.6% (3/35)</td>
<td>14.3% (5/35)</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>97.1% (34/35)</td>
<td>2.9% (1/35)</td>
<td>0/35</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>97.1% (34/35)</td>
<td>2.9% (1/35)</td>
<td>0/35</td>
</tr>
</tbody>
</table>

A, B Indicates significant differences (p<0.001) in prevalence of PCV-2 DNA negative litters within a farm

I, II Indicates significant differences (p<0.05) in prevalence of litters with 2-3 PCV-2 DNA positive piglets within a farm
CHAPTER 5 – GENERAL CONCLUSIONS

1. Systematic review and pairwise meta-analysis of the three commercially available PCV2 vaccines in the United States

Vaccination against PCV2 is commonly used and it is estimated that approximately 99% of the growing pigs in the United States are vaccinated against this virus (Shen et al., 2012a). Despite the widespread prevalence of vaccination, there are currently very few studies comparing the efficacy of PCV2 vaccines, and recent advances in systematic reviews and meta-analyses (Glenny et al., 2005; Sargeant et al., 2006; O'Connor et al., 2010; Dias et al., 2011) have made such methods ideal to determine and compare efficacy. The few previous PCV2 vaccination meta-analyses focused on overall vaccine efficacy (Kristensen et al., 2011), the performance of Ingelvac® CircoFLEX™ when compared to non-vaccinated animals (Holck et al., 2010; Coll et al., 2010), or the comparative efficacy of Ingelvac® CircoFLEX™ against Circumvent® PCV (Diaz et al., 2010). This is the first pairwise meta-analysis of all three vaccines commercially available in the United States.

Within the context of this meta-analysis, Ingelvac® CircoFLEX™ and Circumvent® PCV were demonstrated to significantly increase ADG, regardless of PRRSV status of the herd. Additionally, the limited number of studies available for Suvaxyn® PCV2/Fostera™ PCV most likely affected the heterogeneity and effectiveness of the random-effects weighted model, and there was no significant difference in ADG between vaccinated and non-vaccinated pigs for this product.

A major concern when evaluating literature to be included in the systematic review portion of a meta-analysis is utilizing conference proceedings, or “gray” literature. Conference proceedings can add to the final dataset by avoiding potential inclusion of bias
due to inaccessibility or language barriers (Sargeant et al., 2006). However, limitations of size and inconsistent data reporting can severely limit the usefulness of these studies. Furthermore, it has recently been demonstrated that conference proceeding abstracts are more likely to report positive results than refereed studies (Snedeker et al., 2010) and may therefore alter the outcome of the meta-analysis.

The results of this pairwise meta-analysis demonstrated a relative rate of efficacy for Ingelvac® CircoFLEX™ and Circumvent® PCV, but not a comparative efficacy between products. While this information can be useful, it is not automatically applicable or intended for comprehensive decision making (Dias et al., 2011). The use of a mixed treatment comparison meta-analysis (Dias et al., 2011), analyzing not only vaccination versus non-vaccination, but also different vaccines in a product-to-product comparison while maintaining the individuality of each study (Glenny et al., 2005; Dias et al., 2011), would be an ideal next step for meta-analysis of PCV2 vaccine efficacy. Additionally, with the development of such a model, routine updating every 18-24 months and inclusion of newer or previously excluded products would allow for better insight to current trends. Finally, mixed treatment comparison meta-analysis with regular maintenance would allow for stochastic interpretation of vaccine efficacy, and would be easily applicable to in-depth cost analysis.

2. Studies on PCV2 vaccination of 5-day-old piglets

Because PCV2 vaccination is widely used, producers and practitioners continually try to adjust recommended vaccination protocols to their own farm specific needs, especially with regards to timing and dose (Cline et al., 2008; Jacela et al., 2011; Thacker et al., 2012).
While it is recommended to follow manufacturers’ suggestions and vaccinate at three weeks of age when pigs are typically weaned, an earlier vaccination age would perhaps minimize labor cost and stress; hence, our second study investigated PCV2 vaccination at either 5 or 21 days of age with two different commercially available products followed by experimental challenge to evaluate vaccine performance.

The pigs utilized for this experiment were PCV2 naïve and therefore seronegative at the time of vaccination. Vaccination with the chimeric vaccine resulted in significantly higher sample to positive (S/P) ratios compared to vaccination with the product based on the PCV2 capsid protein, regardless of vaccination age. Similar findings were also observed with the obtained PCR data. One of 16 pigs in the chimeric groups was positive for PCV2 DNA at one of three time points (7, 14, and 21 days) after PCV2 challenge. In contrast, in the subunit groups, at 14 and 21 days post PCV2 challenge, 43.75% (7/16) and 25% (4/16) of the pigs were viremic, respectively.

While we did observe differences in both antibody response and levels of viremia between products, both products were efficacious in the prevention of PCVAD when compared to the non-vaccinated, positive control pigs. After necropsy, post-mortem evaluation of selected lymphoid tissues demonstrated no significant differences between any of the vaccinated groups and the non-challenged negative control group, while there was a significant difference between all vaccinated groups and the non-vaccinated challenged positive control group, demonstrating a reduction in the development of clinical PCVAD. Furthermore, the differences observed between the vaccine product groups were minimal, regardless of vaccination age. This demonstrated that not only were the products efficacious, pig age at the time of vaccination did not alter the efficacy of the product.
Previously, maternal immunity has played a role in vaccine failure against swine influenza virus (Blaskovic et al., 1970; Mensik et al., 1971; Loeffen et al., 2003), though passive immunity has not been shown to affect PCV2 vaccine efficacy under experimental conditions (Opriessnig et al., 2008). For this study, it was important to use naïve pigs due to the difficulties observed with reproduction of PCVAD in seropositive pigs (Opriessnig et al., 2004; Shen et al., 2012b). Furthermore, the use of naïve pigs allowed us to use fewer animals to definitively demonstrate that the neonatal piglet immune system is capable of mounting an appropriate immune response to vaccination, and develops efficient humoral immunity to later challenge against PCV2.

Overall, our results indicated that vaccination at 5 days of age was efficacious and safe, and decreased PCV2 viremia and PCV2-associated lesions in a triple challenge model using PCV2b, PRRSV and PPV.

In light of recent evidence of possible maternal interference under field conditions (Fraile et al., 2012), this early vaccination study would be ideally performed as a clinical trial in the field with a larger cohort from a breeding herd in which the piglets possess detectable and varying levels of maternal antibodies prior to vaccination.

3. Vaccination of sows reduces the prevalence of PCV2 viremia in their piglets under field conditions

Dam vaccination against PCV2 is a method producers would like to use to prevent both vertical and horizontal PCV2 transmission to offspring and reduce or even prevent PCV2 viremia in piglets at an early age and before an active piglet vaccination would work (Opriessnig et al., 2009; Madson et al., 2009a; Madson et al., 2009c; Opriessnig et al., 2010;
Kurmann et al., 2011; Fraile et al., 2012). To better identify the efficacy of dam vaccination, our third study focused on its role in PCV2 prevalence in offspring. Our results demonstrated a lower overall PCV2 viremia in piglets derived from vaccinated dams that had colostrum access prior to serum collection.

Due to variances in farm management practices resulting in blood collection of Farm 1 pigs after suckling and of Farm 2 pigs before suckling, adjustments to the data analysis had to be made. Analysis of the data at the farm level allowed for an evaluation of PCV2 viremia in post-suckle serum and the role of colostrum uptake on viremia in the offspring. It was determined that PCV2 viremia was reduced in offspring of vaccinated dams with colostrum access.

Another interesting observation was the prevalence rates of the two main PCV2 genotypes. We observed equivalent prevalence of PCV2a and PCV2b in contrast to earlier findings of greater prevalence of PCV2b (Shen et al., 2010), indicating a potential ecological shift of the virus on the investigated farms which could be attributed to widespread vaccination (Shen et al., 2012a).

Overall, the results of this study demonstrated that piglets with colostrum access derived from vaccinated dams had significantly higher amounts of anti-PCV2 antibodies than those derived from non-vaccinated dams. Additionally, piglets with colostrum access derived from vaccinated dams had a significantly lower prevalence of PCV2 viremia as opposed to those derived from non-vaccinated dams. Furthermore, there was a significantly higher rate of litters containing one to three PCV2 viremic piglets when they were farrowed from a non-vaccinated sow.
A more geographically diverse field study utilizing dam vaccination that focuses on solely pre- or post-suckle serum collection would allow for better characterization of the potential vaccination influence on the shift in PCV2 prevalence. Additionally, evaluation of different PCV2 vaccines as well as vaccination times relative to estrus and gestation in dams is vital to optimize breeding herd vaccination. Finally, comparing the offspring from vaccinated and non-vaccinated dams from farrow to finish would be ideal to determine if dam vaccination contributes to performance.

4. Final conclusion

In conclusion, PCV2 vaccination continues to be the first line of defense against the development of the economically important disease manifestations comprising PCVAD. We demonstrated that vaccination in either growing age pigs or dams is safe and efficacious, and that the use of PCV2 vaccines increases production parameters. Overall, this thesis contributes to the collective knowledge about PCV2 vaccination and novel methods in their administration.
5. References


Burch, D., 2008. Porcine circovirus vaccines - where are we? Pig Progress. 24, 7-9


