EVALUATION OF PORCINE CIRCOVIRUS TYPE 2 VACCINATION

By

María Cristina Venegas Vargas

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Large Animal Clinical Sciences

2011

ABSTRACT

EVALUATION OF PORCINE CIRCOVIRUS TYPE 2 VACCINATION

By

María Cristina Venegas Vargas

Porcine circovirus type 2 (PCV2) is one of the most important diseases in the swine industry worldwide, having a high economic impact in the sector since its description in the 1990s. The introduction of the first vaccine in 2006 was a major breakthrough for control of the disease, but there are still important questions to be addressed regarding the use and implementation of PCV2 vaccination. In order to answer some of these questions the following studies were performed to observe the effect of PCV2 vaccine on growth performance and carcass composition in herds of different health status and also to evaluate the use of sentinel pigs as a tool for PCV2 detection.

Dedicated to my family

ACKNOWLEDGMENTS

My sincere recognition to Dr. Barbara Straw and Dr. Julie Funk for their advice and support during my masters.

My appreciation to the producers who kindly supported the research

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Literature Review Porcine Circovirus Type 2 (PCV2)

Despite the fact that PCV2 is one of the smallest viruses in nature, it causes significant production losses for the swine industry in the US and worldwide. Major strides in understanding the disease have been made, but there are still gaps in our understanding of the pathogenesis and transmission of the virus, as well as in appropriate control measure—particularly for subclinically infected herds. This review provides an overview of Porcine Circovirus and highlights opportunities for further research.

Agent

Porcine circovirus (PCV) is a small, nonenveloped virus containing covalently closed, circular, single-stranded DNA of 1767- 1768 bp. It was originally recognized as a noncytopathic contaminant of a continuous pig kidney cell line (PK-15) in 1974 (1) (2) (3) (4). Under experimental conditions, this PK-15- derived PCV isolate did not produce disease in pigs. (1) (3). Years later, the presence of a new antigenically and genomically distinct PCV was associated with a disease syndrome in pigs, named postweaning multisystemic wasting syndrome (PMWS) (3) (5). To differentiate these viruses, the nonpathogenic PCV is designated porcine circovirus type 1 (PCV1) and the pathogenic PMWS- associated PCV is called porcine circovirus type 2 (PCV2) (3) (5). Both viruses

are members of the *Circoviridae* family and the genus *Circovirus* (3) (5) (6). Viruses that belong to this family have characteristic virions that exhibit icosahedral symmetry (3). Studies have shown that PCV2 can be separated into two major groups, which are now designated as PCV2 group 1 or PCV2b and PCV2 group 2 or PCV2a (3) (4) (6) (7). The PCV2b genotype can be divided into 3 clades (1A-1C), and the PCV2a genotype can be divided into 5 clusters (2A-2E) (3) (4). The PCV2b isolates had a predominantly European origin, whereas PCV2a isolates had a North American origin (3) (4). However, both types are closely related to each other with 93-100% nucleotide sequence identity (4). Based on restriction fragment length polymorphism (RFLP), PCV2 group 2 is associated with the term *old* 321, 422 and PCV2 group 1 is associated with the term *new* 321 (3). Recombination between the PCV2a and 2b genotypes has been reported (6) (8). PCV2 group 1 has become the dominant strain over time, with a major shift from PCV2 group 2 to PCV2 group 1 which occurred by 2003 (9). The shift from group 2 to 1 as the dominant strain is consistent globally, with the exception of Korea, Japan and Australia (9). The EU consortium on porcine circovirus disease proposes a standardized nomenclature for PCV2-genotypes. It proposes naming the three PCV-2 genotypes PCV-2a, PCV2-b and PCV-2c. This last genotype has to date only been recovered from swine in Denmark, and only when PMWS was not present or at least was undetected (6) (9) (10). PCV contains six open reading frames (11). The two major open reading frames (ORFs) are ORF1, the *rep* gene, which encodes two replicase proteins named Rep and Rep′, both involved in the ssDNA virion replication and ORF2, the *cap* gene, which encodes the capsid protein, considered the most immunogenic

protein (12) (13). A third gene, ORF3, has been recently reported. The product of ORF3 has been found to be involved in apoptotic activity both in vitro, and in vivo mouse models (4) (12).

Industry Prevalence

PCVAD has rapidly become one of the most devastating and economically important diseases for pork producers in North America and worldwide (19) (21).PCV2 is globally distributed and very few herds are PCV2-free (14). It is considered ubiquitous in domestic pigs (4) (6). It has been officially reported in several parts of the world including North America, Europe, Australia, Asia, South America and Central America (4) (15) (16). PMWS has been diagnosed on all five continents that have swine (6). Most swine populations in North America are infected with both PCV2a and PCV2b (18). Depending on the individual farm situation and influenced by intrinsic and extrinsic factors, the percentage of the population affected may vary from 1 to 50% (19). The incidence of disease ranges from 15% to 20% of the total number of infected pigs in those PMWS affected farms (20). Major clinical losses due to PCVAD in US farms have been recognized since late 2005, with mortality rates in growing pigs ranging from 10% to 40% on affected farms, some herds as high as 80% (4) (17).

Clinical signs

The clinical-pathological range of PCV2 includes PMWS, as well as a number of conditions collectively known as porcine circovirus-associated disease (PCVAD) in North America and as porcine circovirus diseases (PCVD) in Europe (3) (6). These conditions,

beside PMWS (now termed PCV2-associated systemic infection in North America) are: porcine respiratory disease complex (PRDC), proliferating and necrotizing pneumonia (PNP), PCV2-associated pneumonia, PCV2- associated enteritis, PCV2- associated reproductive failure and porcine dermatitis and nephropathy syndrome (PDNS) (3) (6) (14) (22).

Post-weaning multisystemic wasting syndrome (PMWS) or PCV2-associated systemic infection

PMWS was first reported in 1995-1996 by Harding and Clark, and now is reported in swine producing countries worldwide (23). PMWS has been reported in pigs 1 to 6 months of age (14) (26) (27), but is most common in 5-6 week old pigs (24) (25).

The clinical signs of PMWS include weight loss or decreased rate of weight gain, unthriftiness, gauntness, pallor and jaundice, enlarged lymph nodes, diarrhea and respiratory distress (3) (5) (11) (23) (24) (25) (27). Gross lesions are not diagnostic; the most common lesions reported are non-collapsed and tan mottled lungs, enlarged lymph nodes (mainly inguinal, submandibular, mesenteric), and in chronic cases, the kidneys may have white streaks or spots (3) (22) (23).Other gross lesions associated with PMWS include reduction or increase in liver size, with orange-yellow discoloration in those cases where icterus is present (22). Less commonly, gastric ulceration of the *pars* oesophagea can also be observed (3) (22).

Porcine Respiratory Disease Complex (PRDC)

PRDC is characterized by a decreased rate of growth; reduced feed efficiency, anorexia, fever, cough and dyspnea (3) (22) (28). PRDC is most commonly observed in

8 to 26 week old pigs and is associated with co-infection with many respiratory pathogens including Porcine Respiratory Reproductive Syndrome (PRRS), Swine Influenza Virus (SIV) and *Mycoplasma hyopneumoniae* (3). There are others pathogens detected in PRDC and studies suggest that PCV2 may play an important role in the PRDC (3) (22) (28).

Proliferative and necrotizing pneumonia (PNP)

The aetiology of PNP remains controversial (71). Initially it was suggested that this condition is the result of a PRRSV and PCV2 coinfection (22). A retrospective Canadian study concluded that PCV2 infection is not essential for the development of PNP lesions, and also demonstrated that PRRSV is consistently and predominantly associated with PNP, and is the key etiologic pathogen (29). But this results compare with two Europeans studies; which suggest a dominant role of PCV2 infection in PNP, insinuate a different aetiopathogenesis for PNP between the two continents (71). In addition to PRRSV and PCV2, SIV and Aujezsky's disease virus (ADV) are suspected to be aetiological agents for PNP (71). A study perform in 2010 in Italian pigs suggests that the major aetiological agents of PNP are PRRSV and PCV2, these results are more in concordance with the Canadian study, in which PRRSV infection was more consistently demonstrated rather than the European studies that suggested a dominant role for PCV2 infection in PNP (71).

PCV2-associated pneumonia

PCV2-associated pneumonia is characterized by lymphohistiocytic to granulomatous interstitial pneumonia, peribronchiolar fibroplasia, and mild-to-severe necrotizing and ulcerative bronchiolitis (3).

PCV2-associated enteritis

It is most common in 8 to 16 weeks old pigs with clinical signs similar to subacute or chronic ileitis associated with *Lawsonia intracellularis*; the intestinal mucosa is grossly thickened, and mesenteric lymph nodes are enlarged (3). Opriessnig et al. (2007) proposed the diagnosis of PCV2-associated enteritis requiring all 3 of the following criteria: 1) diarrhea is present, and 2) Peyer patches (and not other lymph nodes) present characteristic lesions, and 3) PCV2 antigen or nucleic acids are present within the lesions (3).

PCV2-associated reproductive failure

PCV2 associated reproductive failure is characterized by increased abortions, stillbirths and fetal mummification, enhanced preweaning mortalities and increased return to oestrus (3) (14) (30). Its presentation is most common in new farm start-ups with a high proportion of gilts in the herd (3). The heart is the primary site of PCV2 replication in fetuses. The characteristic lesion in stillborn and neonatal pigs from field cases is a nonsuppurative to necrotizing or fibrosing myocarditis associated with abundant PCV2 antigen (3). Given that congenital tremor type AII was linked to PCV2 infection on only one occasion, it is not currently considered a PCVAD syndrome (6) (31).

Porcine dermatitis and nephropathy syndrome (PDNS)

The etiology of PDNS is unclear. Currently there is a hypothesis that PCV2 is the causative agent, but the simple presence of PCV2 in most animals affected with PDNS is not conclusive proof of pathogenesis (32). PDNS has not been experimentally reproduced (3). PDNS affected pigs are typically 6-16 weeks of age (33). The clinical signs are haemorrhagic skin lesions, (especially on the rear legs and perineal area), fever, lethargy, anorexia, prostration, stiff-gait and/or reluctance to move, enlarged tan or pale, waxy looking kidneys with petechial hemorrhages in the cortex, also enlarged and hemorrhagic inguinal, renal, as well as other lymph nodes (3) (32) (33). Spleen infarcts may be also present (27). Histologically, there is a systemic necrotizing vasculitis (22) and glomerulonephritis (33) (3). PDNS is often fatal (3). These lesions are consistent with an immune-complex disorder, suggesting that extremely high PCV2 serum antibody titers and immune-complex deposition may be an important factor for disease (32). Numerous viral and bacterial agents have been implicated as possible causative agents for PDNS (3) (22).

Transmission

PCV2 can be transmitted by direct contact via oronasal, fecal and urinary routes (3) (6) (33) (34), as well as, contaminated fomites and exposure to contaminated feeds (33) or via consumption of tissues from PCV2 infected animals. Horizontal transmission of PCV2 was demonstrated to occur up to 42 days post infection when cesarean derived colostrum deprived (CD/CD) piglets were comingled with infected pigs (3). Infected pigs can transmit the virus to seronegative pigs in separate and neighboring pens (33) (6).

In experimental studies of CD/CD infected pigs, shedding of PCV2 was determined by polymerase chain reaction (PCR). PCV2 was detected as soon as 1 day post-infection in nasal swabs, feces, and oropharyngeal swabs and lasts as long as 70 days post-infection, with the exception of oropharyngeal swabs. In the case of serum and whole blood the virus was first tested at seven days post-inoculation, at which time the virus was detected (3). PCV2 is most likely excreted through respiratory secretions, oral secretions, urine, and feces, as well as in ocular secretions, milk and semen of both Porcine Circovirus Associated Disease (PCVAD)-affected pigs as well as infected but clinically normal pigs. PCVAD affected pigs shed higher viral loads as compared to sub-clinically infected pigs (3) (6). There is experimental and field evidence that PCV2 may establish persistent infection in pigs (34). Vertical transmission has been confirmed in the field and experimentally, resulting in piglets that are viremic or persistently infected at birth (3) (34) (33). Most sows are resistant to infection at sexual maturity (33).

PCV2 has been detected in semen samples. One study suggests the possible intermittent shedding in semen although experimental confirmation of the transmission of PCV2 via artificial insemination is lacking to date (3) (35) (30). In experimentally challenged boars PCV2 has been found to be shed in semen continuously (30). PCV2 DNA has been detected in semen up to 27.3 months post-infection in naturally infected boars (30). The amount of the virus shed in semen is generally in low quantities and serum viremia usually precedes detection in semen (30). Although PCV2a and PCV2b shed in semen were infections in a swine bioassay model (30), the low doses of PCV2 ($10^{5.6}$ -$10^{5.8}$ PCV2 genomic copies per mL) in extended semen used for Artificial

Insemination (AI) appears to be below the infectious dose necessary for eliciting reproductive failure, seroconversion, or PCV2 viremia in naïve gilts and their offspring (30). To the contrary, another study reported an association between reproductive failure and insemination of those dams with PCV2 spiked semen at the same dose (10^4 $TCID_{50}$/mL) which failed to produce clinical signs in the previous study (30). There is certainly a need for further study regarding transmission of PCV2 via semen.

Pathogenesis and Co-infections

The pathogenesis of PCV2 infection and the main cell types that sustain PCV2 replication are still not completely understood (3) (14). PCV2 is an immunosuppressive agent inducing lymphoid tissue lesions and progressive growth retardation in infected pigs (14). Lymphoid depletion and lymphopenia in peripheral blood is a common characteristic in pigs that develop clinical PCVAD (3). Changes in peripheral blood mononuclear cell (PBMC) subpopulations and altered cytokine expression patterns have been reported (27). PCV DNA synthesis depends on cellular enzymes expressed during the S phase of mitosis (12) (36). Large amounts of PCV2 antigen or nucleic acids can be found in the cytoplasm of macrophages and dendritic cells replacing lymphocytes in the depleted follicles in lymphoid tissues (3) (14). The cause(s) of the reduction of lymphocytes in PCVAD-affected pigs is still unknown (3). Virions are assembled in both nuclei and cytoplasm and released from infected cells in the absence of viral cytopathic effects (37). *In vitro* studies suggest that monocytic cells may not represent the primary

target for PCV2 replication (3) (27). It appears that this amount of PCV2 viral antigen is the result of accumulation of viral particles (27).

Some preliminary *in vitro* data have shown that PCV2 enters porcine monocytic cell lines principally via clathrin-mediated endocytosis and an acidic environment is required for this entry (27). Mature dendritic cells (DC) and DC precursors allow PCV2 internalization by non-macropinocytic uptake (27). Pérez-Martín et al. (2007) reported that at least a certain proportion of macrophages may support PCV2 replication, but the main cells where PCV2 replicates are of epithelial/endothelial origin (12) (38). In dendritic cells PCV2 is able to escape lysis (3) and to stay unprocessed in large quantities for a long period of time (14). It has been speculated that the dendritic cells can provide a vehicle for transport of the virus throughout the host (3).

PCV2 infection induces a down-regulation of the production of mitogen-induced interferon gamma, IL-2 and IL-4, IFN-α, TNF-α while stimulating the production of the immune suppressive IL-10 (14) (38). It is known that the DNA of PCV2 can be recognized by the immune system as a danger signal (38). Free viral DNA as well as virus-particle mediated delivery of DNA has been demonstrated to inhibit natural interferon producing cells (NIPC) function (39). The important role played by NIPCs in antiviral innate immunity, may indicate that viral inhibitory activity is a key event in the pathogenesis of PCV2 diseases (39). Results *in vitro* suggest the potential of PCV2 to suppress Th1 responses and that PCV2 has the potential to act as an immunosuppressive/immunomodulating virus (38). Virus-like particles based on PCV2 Cap protein are also capable of stimulating cytotoxic T cells suggesting that those

PCV2-specific T cells can recognize antigen processed from VLPs as well as from live virus (38). It is suggested that the clearance of PCV2 infection occurs by the classical combination of cell-mediated responses, measurable as IFN-γ-secreting cells, together with a significant neutralizing antibody production (38). Failure in one or the other or both responses is been hypothesized that might result in PMWS development (38).

It is clear that the immune system plays a central role in the pathogenesis of PMWS (36). What is unclear is why only a proportion of PCV2 infected pigs develop the disease and what mechanisms lay behind it (36). In fetuses, PCV2 seems to replicate in tissues or cell types with a high-mitotic rate such as fetal myocardiocytes (27) (36). PCV2 is able to replicate in *in vivo* produced zona pellucida-free morulae and blastocysts, suggesting a potential effect of PCV2 in embryonic stages (27). Porcine Dermatitis and Nephropathy Syndrome (PDNS) is considered to be a type III hypersensitivity reaction, but the instigating antigen is still unknown. PCV2 antigen has been speculated to be responsible, but experimental proof is still lacking (27) (4).

PCVAD is multifactorial in causality and not all pigs that are infected will develop clinical PCVAD. PCV2 is considered a necessary but not sufficient factor to develop PMWS (22) (40). Krakowka at al. (2001) confirmed in gnotobiotic experiments that PCV2 alone is responsible for the PMWS (41).

The outcome of PCV2 infection is influenced by different extrinsic and intrinsic factors: virus, host, coinfections, and immune modulation (3), immune status of sow, timing of PCV2 infection and management-related factors (Table 1.1) (6).

Immune status of the sow

Maternally-derived immunity against PCV2 can protect pigs against development of PMWS, and this protection is considered to be antibody-dependent (6). It has been reported that piglets from sows with low antibody titres to PCV2 have higher mortality and PCV2 viraemia in sows has been associated with piglet mortality (42) (6). One study demonstrated that circumstances leading to the expression of the clinical disease are highly dependent on the dam's characteristics; for example passive immunity and general health status (43). The use of a killed PCV1-2 chimeric vaccine has been demonstrated to reduce viremia and prevents microscopic lesions associated with PCV2 in the presence of maternal antibodies (44).

Timing of PCV2 infection

Some findings suggest that the earlier PCV2 infection occurs, the higher the risk of pigs developing PMWS (6) (43) (45), but a recent study did not find any association between the age at PCV2 infection and the consequent development of PMWS (6).

Virus

Studies confirmed that minimal changes in the genome of PCV2 isolates can markedly alter their virulence (3) and pathogenicity (4). It is suggested that there is an association between novel epizootics and emergence of a new variant of PCV2 (6) (4). Epidemiological studies indicate that genotype PCV2b is currently the most prevalent in naturally occurring infections and there are indications that PCV2b may be more virulent than PCV2a, due to the associations that have been made between PCV2b and PMWS-affected farms and between PCV2a and non-affected farms (6) (13). Only PCV2a has been identified in Australia, where PMWS has not been reported (6). The presence of

more than one strain of PCV2 existing at the same time in pigs has recently been confirmed; this from the tissue of commercial affected pigs submitted to a veterinary diagnostic laboratory (4).

Host

Pigs of all breeds appear to be susceptible to PCV2 infection, and clinical PCVAD has been occurred in a wide variety of purebred and crossbred pigs (3). Field observations suggest that animal susceptibility to PMWS varies with the boar-line used (6) (8). It has been reported that Purebred or cross-breed Piétran pigs has lower mortality by PMWS than Large White-Duroc cross pigs (4). But another study didn't find any breed-related difference in mortality (6) (3). In experimental investigations Landrace have a higher predisposition to PCV2- associated disease as compared to Duroc, Large White and Piétran pigs (6) (46) (4) (3). Another field study demonstrated an increased mortality in the offspring of a 25%Large White/75%Duroc paternal line compared with the offspring of 2 other lines (100%Pietran, 50% Large White/50%Pietran) (3). Although field studies have indicated that castrated males are more susceptible than females (46) this difference could be due to castration rather than gender (6).

Coinfections and immune modulation

There is field and experimental proof that coinfecting pathogens trigger PCV2-infection to full development of clinical disease (37). Early in the discovery of PMWS, it was believed that another agent was required for PCV2 to cause disease. This was until Magar et al. (2000) and Krakowka et al. (2001) demonstrated that PCV2 could

independently cause PMWS (47) (41). Experimental coinfections of pigs with PCV2 and other viruses such as porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV) or bacteria such as *M. hyopneumoniae* have been demonstrated to increase the amount of PCV2 viral load and PCV2-associated lesions and to enhance the incidence of PCVAD (3). The rates of occurrence of coinfecting pathogens in PCVAD as consolidated values reported by Ramamoorthy and Meng (2010) based on four retrospective independent studies (field cases submitted to a diagnostic laboratory) include PRRSV (41%), *M. hyopneumoniae* (27%), bacterial septicemia (10%), bacterial pneumonia (6%) and SIV (4%); only 1% was caused alone by PCV2 (4). Coinfection with Aujeszky's disease virus has also been reported (3) (37). A retrospective study performed in the USA reported that in cases of PCV2 coinfection with bacterial septicemia the most frequently pathogen isolated was *Streptococcus suis*, and in the cases with bacterial pneumonia the most prevalent coinfection pathogen was *Pasteurella multocida* (48). In Korea *Haemophilus parasuis* was the most prevalent bacterial co-infection (11). There is field and experimental evidence of coinfection of PCV2 and Porcine torque teno virus (TTV), PCV2 and Porcine epidemic diarrhea virus (PEDV) and PCV2 and *L. intracellularis*. There is also field evidence of coinfection of PCV2 and *Pneumocystis carinii*, as well as PCV2 and *Cryptosporidium parvum* (37). Some reported coinfections such as *Aspergillus* spp., *Candida albicans* or *Chlamydia* spp. are most likely secondary to the induced immunosuppression (6).

PRRSV is most frequently associated with PCV2 in PMWS cases; coinfections with PRRSV increased the risk of PCVAD by several fold and PCV2 infection has been

implicated in the reduction of PRRSV vaccine efficacy (4). PRRSV also has a strong association with PDNS and necrotizing pneumonia (4). Timing of coinfections may be an important factor and probably needs to be further investigated (37). Opriessnig et al (2007) suggests it is more likely that several known and unknown pathogenic and nonpathogenic organisms that vary from region to region may be able to trigger progression of PCV2 infection to PCVAD (3). Immune stimulation may trigger progression of PCV2 infection to diseases and lesions characteristic of PCVAD (49) (3). The immunomodulation that occurs due to *M. hyopneumoniae* vaccination or infection increase the incidence of PMWS. This has resulted in the recommendation to vaccinate pigs 2 weeks prior to expected PCV2 exposure to mitigate this effect (4).

Co-infecting pathogens may increase PCV2 in two ways: by inducing release of host IFN-γ in response to the co-infecting pathogen and by beginning host cell replication especially of cells of the immune system enhancing PCV2 replication (37). There is evidence that immune stimulation by vaccines against *M. hyopneumoniae, Actinobacillus pleuropneumoniae*, PRRSV and classical swine fever virus may enhance PCVAD (6) (48) (49) (3). Also the type of vaccine used, the timing of injection of adjuvanted vaccines and the age of the animals at the time of vaccination may influence the effect of PCV2 infection (49) (3). There is conflicting evidence that oil-based adjuvants accentuate this immune stimulation (6). Some studies used adjuvants like Freund's adjuvant and keyhole limpet hemocyanin to be able to reproduce the full spectrum of PMWS (4) (41). The difficulties in reproducing PMWS using PCV2 alone and its ubiquity results in a hypothesis that PMWS may be triggered by an unknown

pathogen, popularly named “agent X” (6). In the case of PCV2 infection, no such unique stressor or molecular mechanism of immune suppression or stimulation has been identified (6) (4). The success of PCV2 vaccines is further evidence that PCV2 is the essential infectious agent of PMWS (6) but we need to remember that PCV2 is a necessary but not sufficient to cause disease.

Immunology

The molecular mechanisms of PCV2 induced immunomodulation are not known but it is independent of virus replication (40). Moreover, viral DNA, but not the PCV2 capsid protein, influences monocytic and dendritic cell responses (40). It has been hypothesized that the PCV2 genome may carry DNA sequences capable of modifying dendritic cell (DC) function, and therefore influences immune defense development (40). Immunomodulatory sequences around CpG motifs (Cytosine phosphodiester Guanine olygodeoxynucleotides) have been identified within the PCV2 genome (40) (38), but a strong immunosuppressive activity is most apparent in the presence of the whole virus genome or the circular double-stranded replicative form of the virus DNA genome (40). The target for PCV2 induced immunomodulation centers on the ability of DC to recognize “danger” signals (40). In experimentally infected pigs, with and without clinical disease, seroconversion to PCV2 has been demonstrated to occur between 10 and 28 days post-inoculation (DPI) (40) (38) (27). Some studies suggest that PMWS affected pigs seroconvert later or have lower antibody titres at 21 DPI than pigs subclinically infected with PCV2 (40) (38) (27). Immunoglobulin isotypes and virus

neutralizing antibody (NA) titres followed the total antibody response; in PCV2 subclinically infected pigs, NA seroconversion occurred from 15 DPI onwards (40). In another study NA were not detected until day 28 DPI (27). A decrease in virus load was found to coincide with the increase of NA titers (40) (50). PMWS has been reported to occur in animals with no or low NA against PCV2 (50), supporting the theory that accumulation of high virus titres occurs in pigs with poor production of antibodies against PCV2 neutralizing epitopes (40). NA appears to be crucial in decreasing PCV2 replication and preventing clinical disease (51). Maternal antibodies decay over a wide window of time (2 to 15 weeks of age); depending in the initial concentration of maternal antibodies (44) (14). Opriessnig et al. (2004) reported a mean PCV2-antibody half-life in piglets, based on declining ELISA S:P ratios of 19 days (95% confidence interval 17.6 and 20.4 days) (34). Meanwhile Lyoo et al. (2010) reported a mean IFA antibody half-life of 18.3 days (95% CI: 15.5-21.1 days) (17). PMWS is rarely observed in pigs younger than 4 weeks of age (27) (38) (40) which may be a result of protective maternal immunity (27) (40). Under field conditions seroconversion occurs after the decline of the colostral antibodies, which decline during the lactating and nursery periods, reaching their lowest levels by the mid-to-late nursery and early finishing periods (27) (40). This seroconversion usually occurs between 7 to 15 weeks of age, depending of the farm. The antibodies may last at least until 28 weeks of age (38) (27) (40). A variable percentage of growing or finishing pigs can be seropositive but viremic, suggesting that the PCV2 antibodies alone might not be fully protective or insufficient to clear the infection (27) (38). In field conditions, adult pigs can be infected but do

exhibit detectable clinical signs (38). Whether this is due to humoral immunity to PCV2 or natural age-resistance is not presently known (27). PCV2 infection generally results in marked antibody production (14). In controlled laboratory conditions, there is a relationship between the level of antibody and protection against challenge (14) (4). The relationship of antibody titer thresholds and protection against disease is currently unknown under natural conditions (14) (40). There is less information regarding the role of the cell-mediated immune response. Three experimental studies which could not reproduced PMWS (PCV2 infected pigs), suggested that the development of IFN-γ secreting cells (IFN-γ-SC) may be a key component of the developing anti-PCV2 adaptive cellular response (40). An important observation was that the reduction in PCV2 load in the blood coincided with the appearance of both specific antibody (especially NA), and PCV2 specific IFN-γ-SC (40). Lymphocyte depletion together with histiocytic infiltration are the most prominent characteristic of PMWS-affected pigs (40). The main cellular changes in lymph nodes are: decrease in follicular DC, interdigitating cells, interfollicular lymphocytes, B cells and CD45RA+ cells (40). There are also changes in the peripheral immune cells: PMWS-affected pigs show lymphopenia involving B-lymphocytes, all T lymphocytes sub-populations, and NK cells, while monocytes and neutrophils increased, with an inversion of the lymphocyte/neutrophil ratio (40). PCV2 by itself is able to alter the cytokine responses of pig dendritic cells and peripheral blood mononuclear cells regardless of the immune or disease status of the animal (38). Since PMWS is a multifactorial disease the final outcome will be the result of the combination of one or more extrinsic or intrinsic factors (38). The precise reasons

a given pig infected by PCV2 develops PMWS or a subclinical infection are still unknown (38).

Diagnosis

The following is the PCVAD **case definition** proposed by the American Association Swine Veterinarians (AASV), which defined the minimum findings necessary to associate a given clinical presentation with exposure to PCV2 (52).

PCV2 can be subclinical or include one or more of the following clinical manifestations concurrently:

1. Multisystemic disease with weight loss (formerly known as PMWS)
2. High mortality: Doubling of historical mortality rate without introduction of a new known pathogen
3. Respiratory signs including pneumonia
4. Porcine Dermatitis and Nephropathy Syndrome (PDNS)
5. Enteric signs including diarrhea and weight loss
6. Reproductive disorders including abortions, stillbirths and fetal mummification (diagnosis requires the presence of fetal myocarditis associated with PCV2 antigen in lesions)

PCVAD is a broad categorization of multisystemic diseases that are confirmed by documentation of the following histopathological findings in affected pigs:

1. Depletion of lymphoid cells in lymphoid tissues of the growing pigs.

2. Disseminated granulomatous inflammation in one or more tissues (e.g. spleen, thymus, intestines, lymph nodes (sternal, bronchial, inguinal and mesenteric), lung, kidney, liver, tonsil, etc).
3. Detection of PCV2 within the lesions of growing pigs.
4. PCV2 associated reproductive disease diagnosis requires the presence of PCV2 antigen in fetal myocarditis lesions.

On a herd basis, there is a distinction between sporadic PCVAD and PCVAD manifested at a level that is considered a herd problem (3). PCVAD diagnosis on a herd basis takes the following into account: the percentage of pigs diagnosed with PCVAD from all submitted pigs and an increase in mortality on the particular farm investigated (3). There are 3 definitions at the herd level:

- Herd Problem: PCVAD is diagnosed in 50% or more of a representative sample, plus a significant increase in mortality compared to previous herd parameters (increase of equal to or more than the mean of historic levels plus 1.66 times the standard deviation, or an increase in mortality that exceeds the national regional level by 50%).
- Sporadic: PCVAD is diagnosed in less than 50% of the representative sample with a concurrent increase of mortality or PCVAD is diagnosed in more than 50% without a concurrent increase in mortality (3).

Subclinical PCV2 infections: PCV2 is present but not responsible for the disease observed in the pig; e.g. low amounts of PCV2 antigen associated with no to minimal lesions (3).

Diagnostic tools

Detection of PCV2 nucleic acid or antigen in clinically healthy pigs or diseased pigs without clinical signs and gross lesions consistent with PCV2 should be interpreted with caution since subclinical PCV2 infection with viremia occurs on almost all farms (27).

Several methods have been developed to detect PCV2 in tissues and to correlate its detection with the presence of lesions. In Situ Hybridization (ISH) and immunohistochemistry are the most extensively used tests for the diagnosis of PCVD (27) (23) and are considered the gold standard for detecting PCV2 (3). For all diagnostic tests, it is critical that there be lesions, because virus may be present in the absence of disease. There is a strong correlation between the amount of PCV2 nucleic acid or antigen present and the severity of microscopic lymphoid lesions in PMWS (27) (22). Techniques that allow the quantification of virus in tissues and/or serum, such as quantitative PCR methods, antigen capture ELISA and immunocytochemical analysis of cryostat sections are useful for diagnosis (27). Serology is best used on a herd basis to determine the time of PCV2 infection by sequential or cross-sectional analyses of the population (3). Serology is not useful for individual diagnosis as the presence of antibodies or antigens is not synonymous with disease.

Detection of anti-PCV2 antibodies:

Indirect fluorescent antibody (IFA) assay

Indirect Fluorescent Antibody assays detect the ability of serum antibody to bind to a fixed monolayer of virus-infected cells. The specific antibodies are labeled with fluorescein-conjugated anti-swine-IgG antibody (53). Studies have shown that there is a

low level of cross-reactivity between PCV1 and PCV2 on the IFA test (3). Limitations of this test are that it is not automated and is based on a subjective assessment (3). An IFA assay based on the entire PCV2 virus had a 57.1% relative sensitivity as compared to an ORF2 protein-based IFA assay (3).

Indirect immunoperoxidase monolayer assay (IPMA)

The IPMA is similar to the IFA with the exception that the antibody used is a peroxidase-conjugated anti-swine IgG (53). In general, IPMA results in higher titers than IFA, and paraformaldehyde used as fixative results in higher titers than acetone or ethyl alcohol (3) (53). Limitations are that it is not automated, and end points determined subjectively (3).

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a sensitive technique for detecting and measuring serum antibodies (3). An ORF2 protein-based ELISA has been described with a relative sensitivity of 98.2% and specificity of 94.5% compared with IPMA (54). Compared with indirect immunofluorescent assay, the diagnostic sensitivity, specificity, and accuracy of PCV2 and ORF2 ELISAs were similar (>90%) (55). In Europe there are commercially available IgG and IgM PCV2 ELISAs. Combined IgG and IgM values might be useful to determine the timing of PCV2 infection.

If the IgM value is ≥ the IgG value, it indicates an early active infection (within the first 21 DPI). If the IgM value is < the IgG value, it is indicative of an active infection (approximately between 20 to 50 DPI) and high IgG values and negative IgM

values are typical of a late or resolving infection (approximately 2 months after infection) (53) (3).

In Europe there is a commercially available competitive ELISA specific for PCV2 antibodies that can be used to detected PCV2-specific antibodies in feces (53) (12).

Serum-virus neutralization (SVN) assay

The serum-virus neutralization assay (SVN) detects the presence of antibodies that have the ability to prevent virus from attaching to and/or infecting cells (53). Neutralizing Antibodies were detected between 15 and 28 days post PCV2-infection and were correlated with protection or clearance of PCV2 infection in gnotobiotic pigs (53) (3). SVN requires either fluorescent antibody or immunoperoxidase staining at the end of testing to determine the presence or absence of virus replication. It requires significant resources and well-trained personnel due to the requirements to synchronize the cells cycle (3).

Detection of PCV2 nucleic acids:

Polymerase Chain Reaction (PCR)

There are several PCR assays described in the literature. Variants of the regular PCR include: Multiplex PCR, Nested PCR, Multiplex-nested PCR, Quantitative real time PCR, and Reverse transcriptase (RT-) PCR. The amount of PCV2 nucleic acids in serum and tissues has been demonstrated to be predictive of the clinical outcome which might be useful. PCR results are reported as negative; positive, no PCVAD ($<10^6$ PCV2 DNA copies); positive, PCVAD suspect ($>10^6$ and $< 10^7$ PCV2 DNA copies); or positive, PCVAD (10^7 PCV2 DNA copies or greater) (56) (3).

In Situ Hybridization (ISH)

In Situ Hybridization (ISH) for PCV2 uses a labeled DNA probe that corresponds to a specific portion of the PCV2 genome (3). It is performed on paraffin-embedded, formalin-fixed tissues (23). ISH has been developed that can detect multiple viruses within the same tissue section (PCV1/PCV2, PCV2/PRRS and PCV2/PPV) (3).

Detection of PCV2 virus or viral antigen:

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) uses a monoclonal or polyclonal antibody to detect PCV2 antigen in formalin-fixed, paraffin-embedded tissues sections (3) (23). A minimum viral load of 10^8 PCV2 genomes per 500 ng DNA is required to result in visible staining using IHC (57) (3).

Virus Isolation

PK-15 cells support PCV2 replication in vitro, and these cells can be inoculated with body fluids or homogenate from pigs suspected to be infected with PCV2 (57) (3). Immunofluorescent or immunoperoxidase staining has to be performed because a cytopathic effect is typically not observed (57) (3). Another version of VI is quantitative virus isolation. In this assay 10-fold dilutions of clinical specimens (serum, tissue homogenate) are inoculated on PK-15 cells; infected PCV2-cells are assay using an indirect immunoperoxidase staining procedure and a PCV2-specific monoclonal antibody. This test has been found useful in discriminating subclinical PCV2 infection from clinical infection (57) (3).

Indirect and Direct Fluorescent Antibody Assays (IFA/FA) on tissue sections

Indirect (IFA) and Direct Fluorescent Assays (FA) use a monoclonal antibody or polyclonal antiserum to detect antigen(s) in frozen tissue sections. It is a rapid assay but the antigen cannot be confidently associated with lesions and the assay is relatively subjective (57) (3). Studies using polyclonal antisera and monoclonal antibodies against PCV1 and PCV2 isolates on cells infected with either PCV1 or PCV2 have shown that there was no cross-reaction (57).

Antigen-Capture ELISA

Antigen-capture ELISA (AC-ELISA) is based on tissue homogenates and is found to be comparable to quantitative virus isolation and IHC. An AC-ELISA has been developed for detection of PCV2 antigen in swine feces (57) (3).

Electron microscopy (EM)

Electron microscopy is used to demonstrate circovirus-like particles directly within a cell and to study the virus structure and size. The overall sensitivity is low, and has a detection limit of 10^5 virus particles (57) (3)**.** EM is more commonly used in research and experimental settings.

Characterization of PCV2 isolate:

Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) uses restriction enzyme digestion of viral nucleic acid (partial or whole) which results in a specific cutting pattern that is visualized on a gel. If there are differences between viruses at the site of enzyme cutting, different patterns can be observed (58). An ORF2 based PCR-RFLP assay using *Hinf*I, *Hin*P1I, *Kpn*I, *Mse*I and *Rsa*I enzymes is able to distinguish among

PCV2 isolates (PCV2A, B, C, D, and E). There is also a PCR-RFLP assay using *Nco*I enzyme that differentiates between PCV1 and PCV2. Another ORF2 based PCR-RFLP is able to distinguish 9 different PCV2 genotypes using the restriction enzymes *Sau*3AI, *Ban*II, *Nsp*I, *Xba*I and *Cfr*I (58) (3).

Sequencing

It is possible to sequence the entire PCV2 genome or to sequence only ORF2 (cap gene) to further investigate possible differences among PCV2 isolates (58) (3). Sequencing results may be a useful epidemiological tool but the knowledge to determine virulence based on sequence information is not yet available. Both techniques are offer by veterinary diagnostic laboratories.

Appropriate diagnostic samples

As previously mentioned a diagnosis of PCVAD cannot be confirmed without evaluation of microscopic lesions and demonstration that PCV2 is associated with the characteristic lesions (3). In order to accomplish this, submission of formalin-fixed tissues to a diagnostic laboratory is required; the preferred specimens are small portions of lung, lymphoid tissues (tonsil, thymus, spleen, ileum, enlarged lymph nodes), liver, kidney, and pancreas (23). If PCV2 isolation is desired, chilled samples of fresh lung, tonsil, thymus, spleen, ileum, enlarged lymph nodes, liver and kidney should also be submitted (23).

Prevention and Control

Management Practices

Prior to the availability of vaccines the treatment and control of PCVAD was focused on management practices that minimize stress, eliminating or reducing coinfections and other factors that could trigger progression of PCV2 infection to PCVAD (3). The implementation of “Madec’s 20-point plan” has been effective in decreasing mortality on affected farms (6). This plan has been summarized as the 4 golden rules: limiting pig-to-pig contact, reduction of stress, good hygiene and good nutrition (6). Recommendations include: all-in-all-out pig flow procedures, disinfection, limited animal contact, mixing of batches and cross-fostering, the isolation or euthanasia of diseased pigs, the maintenance of appropriated temperature, air-flow and stocking density, and appropriate use of anti-parasitic treatments and vaccination (6). Regarding disinfection, due to the absence of an external envelope PCV is very stable in the environment and resistant to many disinfectants. It is resistant to low pH (pH 3), chloroform and temperatures of 70ºC for 15 minutes (4). PCV2 is also resistant to lipid-dissolving disinfectants, such as those based on alcohol, chlorhexidine, iodine and phenol. However, PCV2 can be inactivated by alkaline disinfectants (sodium hydroxide), oxidising agents and by quaternary ammonium compounds (6) (59). There is the suggestion that spray-dried plasma products (included in swine feeds as a highly digestible protein source) could potentially contain residual viable virus (6). Concerning the control of coinfections, care is required to evaluate the use of vaccines as potential disease exacerbation could result from their immunostimulatory effects. This can be

ameliorated by management of the timing of vaccinations within herds (6). As an example, it has been recommended that *M. hyopneumoniae* vaccine administration should occur between 2 and 4 weeks prior to expected PCV2 exposure, with the purpose of avoiding enhancement of PCV2 replication (6) (3). Other recommended prevention or control measures include changes in boar usage (since differences in the susceptibility of some breeds and lines have been reported) in herds with severe forms of PMWS (6). It is also suggested to use boars that are free of major pathogens, including PCV2 (6). The addition of anti-oxidant feed additives, conjugated linoleic acid and spray-dried plasma to the ration fed to nursery pigs can ameliorate the clinical effects of PMWS (6). There is evidence that the administration of immunomodulatory phytosterols reduces the production losses on PRDC and PMWS affected farms (6). In a study, that used a modeling approach to assess the influence of several management practices within a farrow-to-finish farm on the age at PCV2 infection, they found that the risk of early infection was significantly reduced when mixing of piglets was reduced at different stages, that sow-targeted vaccination delayed the infectious process until the waning of passive immunity and piglet-target vaccination decrease the total number of infections (45). The model also suggested that changing from a low prevalence of PCV2 infected semen to a higher one significantly increased the risk of early infections. Two levels of prevalence of infected semen were tested in the model: 3.3% and 18.2%. (45).

Vaccination

In the USA, PCV2 vaccination was introduced in 2006 and it is estimated that approximately 99% of all growing pigs in USA are now vaccinated with a commercial vaccine (60). There are four commercial killed vaccines against PCV2 available in different regions of the world (4) (40) (61); of them, one inactivated PCV2 vaccine (Circovac®, Merial Animal Health) has been designed for use in healthy female breeding age pigs. Circovac®, it is indicated for use in sow and gilts 2-4 weeks prior to farrowing (40) (61). It is not currently available in the USA.

For growing pigs there are two different commercial vaccines, based on an ORF-2 protein expressed in a baculovirus vector (40) (61) (60). Of them, one is a single dose application (Ingelvac® CircoFLEX™, Boehringer Ingelheim) and the other one requires two injections (Circumvent® PCV, Intervet-Schering-Plough Animal Health) 3 weeks apart. Both are licensed for healthy pigs 3 weeks of age and older (61). There is another vaccine that is based on a PCV1-2 chimeric virus (ORF-1 from PCV1 and ORF-2 from PCV2, Suvaxyn®PCV2 One Dose, Fort Dodge) (40) (60) (61). As a precautionary measure Suvaxyn® PCV2 One Dose has been temporarily removed from the market due to a chimeric PCV1/2a (whose genome is composed of the ORF1 of PCV1 and the ORF2 of PCV2a) isolate identified in Canadian pig tissue homogenates that were harvested from a commercial pig (60) (62). It is single dose and licensed for healthy pigs 4 weeks and older (61). All four vaccines are based on genotype PCV2a strains but all have been shown to be effective in preventing lesions and disease associated with either PCV2a or PCV2b (61) (40). They are also successful in reducing morbidity and mortality in herds with PCVAD (51). It is recommended to vaccinate at least 3-4 weeks ahead of

anticipated exposure (19). The four vaccines have all been proved effective under both experimental and field conditions (40) (61) (6); the following benefits for animal health and growth performance have been attributed to the usage of PCV2 vaccines:

Under experimental conditions:

PCV2 vaccines reduce PCV2 viremia in serum after PCV2 challenge (6). A study from Kansas State University PCV2 Team reported that vaccination of pigs resulted in a ten-fold reduction in virus load compare to controls (8). They also reduced PCV2-associated lymphoid lesions (6); this has also been reported in field vaccination studies (40). Other benefits are reduction of the amount of PCV2 DNA/antigen in tissue, reduce nasal shedding of PCV2 DNA, and reduce the fecal shedding of PCV2. Furthermore, PCV2 vaccines induce the production of anti-PCV2-IgM antibodies, anti-PCV2-IgG antibodies, anti-PCV2-neutralizing antibodies and there is cross-protection between PCV2a and PCV2b (61).

Under field conditions-growing pigs:

PCV2 vaccines improve average daily gain (6) (40), increase percentage lean meat yield, (although studies are not consistent in the effect for this outcome (63)). The feed conversion is also improved (6) (40). Furthermore, PCV2 vaccines decrease mortality rate (40) (17), and also back fat depth, but the literature has varied responses for this outcome (63). In addition, PCV2 vaccines increase the numbers of closeouts, reduce the medication cost (40) and reduce the number of coinfections (6) (64) (61).

Under field conditions- breeding animals:

PCV2 vaccines reduce the numbers of mummies, increase the numbers of live born pigs, increase the numbers of pigs per sow per year and reduce the numbers of open days per sow per litter (61).

Usually pigs with low amounts of anti-PCV2 antibodies at the time of vaccination show a response to vaccination at approximately 2-3 weeks post vaccination (61). The mean IFA antibody half-life and the SN antibody half-life of the vaccinated groups in a study that compared the efficacy of three commercial PCV2 vaccines in conventionally reared pigs was significantly longer than that of the non-vaccinated control group and the IFA titers in the three vaccinated groups also decreased more slowly than in the control group (17). Moreover the mean IFA titers were significantly higher than the control group at 12 and 15 weeks of age, and the SN titers were significantly higher at 6, 9 and 12 weeks of age (17). The group that received the two dose vaccine had significantly higher titers than the other 2 vaccine groups (17). No all pigs seroconvert against PCV2 after immunization with a chimeric virus, but the absence of seroconversion the vaccinates were still protected from challenge infection in terms of developing PCV2-viremia and clinical signs (40) (61). It is generally believed that the success of PCV2 vaccines is due to induction of a strong cellular immune response (61). Kekarainen et al. (2010) mentioned that maternal antibody can interfere with seroconversion following vaccination, although no effect on vaccine efficacy was apparent (40). There is one group that reported that pigs with IFA titer ≤ 1:320 being easily immunized and those with greater titers less predictably (8). On the other hand, there are controlled research trials that have shown that maternal anti-PCV2 antibodies

do not interfere with PCV2 vaccine efficacy (61) (44). Vaccination of growing pigs with the same vaccine used in the dams did not negatively influence vaccine efficacy as measured by amount of PCV2-DNA in sera, tissue load of PCV2 and histological observations (21). There have been increasing reports of apparent vaccine failure in late finisher pigs (19). There has generated concerns about the duration of PCV2 vaccine-induced immunity (19). Potential reasons for vaccine failures include: failure in compliance with proper vaccine protocols, failure to use the recommended dosage, vaccination of sick and immunocompromised pigs, or the presence of interfering passively acquired antibodies (although the last reason is in doubt as previously mentioned) (19). Other potential reasons for vaccine failure are differences between the vaccine strain and the field strain, adjuvant type, amount of PCV2 antigen in the vaccine preparation and administration regimen (19).

Cost of PCVAD and Benefits of Control Measures

The losses produced by PCVAD are not only a result of the clinical illness and mortalities but also from subclinical infection. When a mild clinical presentation or subclinical form of PCVAD is reported, the impaired growth performance, not mortality, is the primary factor that affects profitability (65). It has been widely reported that PCV2 vaccination has a substantial economic impact on performance measures other than mortality rate (46). Diminished cull rate and increased prime market rate are reported (66). A consultancy in the UK created an online cost/benefit calculator for vaccine use to be used by producers or veterinarians (67). One trial in the USA reported

an estimated $4.38 return on investment (ROI) for every dollar invested in a particular commercial brand vaccine (65). Even though PCVAD is one of the most devastating and economically important diseases in North America, vaccination has been shown to be effective in combating PCVAD, as several field investigations have clearly demonstrated the efficacy of the current commercial PCV2 vaccines (19).

Carcass composition

The value of a pig carcass is determined by its weight, fatness level and muscularity, although quality of the muscle mass should also be considered (68). Backfat depth is often the major criterion of assessment of quality in pig carcasses at the point of payment to the producer (69). Dressing percentage is the ratio of dressed carcass weight to the weight of the live animal, expressed as a percentage (69). In addition to genetic factors, there are many environmental factors that interact and influence meat quality: feeding, pre-slaughter handling, stunning, others (68). Research in several species indicates that an animal's immune system response to disease organism antigens is a major cause of reduced growth rates (70).

PCV2 as an immunosuppressive agent can allow for infection of pigs with secondary pathogens, which increases the possibility of morbidity which can impact the metabolism of formation of muscle and fat deposition, the main components of the carcass measurements used to determine the value of the carcass in the market for producers. So the implementation of PCV2 vaccine, as a consequence of reduced negative effects on growth and clinical illness, may also influence carcass traits.

What little that has been publishing regarding the influence of PCV2 vaccination on carcass composition is contradictory. There are reports that PCV2 and *M. hyopneumoniae* vaccinated pigs have heavier carcass weight than non-vaccinated pigs (28) (65) (72). Also, PCV2 vaccinated pigs had greater loin muscle depth (65). But it was also reported that *M. hyopneumoniae* vaccine didn't have any effect on loin eye area, backfat thickness, and index (72), and PCV2 vaccine did not have effect on lean meat ratio (28), percentage lean, loin depth and backfat after adjusting to a common carcass weight (63)

PCV2 is one of the most important viral pathogens in the swine industry. Currently, most producers manage PCV2 on their farms through a combination of vaccination and management practices.

Due to the clinical improvements seen with PCV2 vaccination, producers and veterinarians are starting to question if, once clinical symptoms have been reduced to a level of non-detection, it could be possible to end the vaccination without negative effects. There is the assumption that vaccination could minimize viral shedding and reduce environmental contamination to a no-effect level; allowing elimination of vaccination programs (46) (79). In order to remove vaccination, it is necessary to evaluate virus shedding in swine herds. Due the documented reduction in viral load in the MSU herd following vaccination, it was to investigate the use of sentinels vs. vaccinated for determination of viral load in vaccinated swine herds with not clinical signs of PCVAD.

It is important to measure pig growth efficiency, but measures of efficiency should include the amount of lean edible pork produced and how desirable it is for processing and consumption (73). If vaccination impacts carcass composition, this information is necessary to completely determine cost-benefit ration of employing vaccine. The immune response to pathogens is a major cause of the reduced growth rate as indicated by studies in several species (74) (75). Disease suppresses appetite and therefore growth rate, which negatively impacts profitability. Because of all the potential impacts on carcass composition, studies of vaccine effectiveness should include carcass measurements. Moreover, due to the common coinfection describe in pigs diagnosed with PCV2 infection; with pathogens such as PRRS and *M. hyopneumoniae* (3) (6) (48) (64) (76) (77) (78). Let to the hypothesis that one potential explanation for the discordance in the literature regarding the effect of PCV2 vaccination on carcass composition may be the difference in health status of the pigs receiving the PCV2 vaccine. With this in mind, the objective of the following studies to evaluate the effect of PCV2 vaccination on ADG, mortality rate and carcass composition in commercial herds with and without PRRS and *M. hyopneumoniae*.

APPENDICES

APPENDICES

Table 1.1

A summary of the management factors influencing the risk of development of PMWS

	Factors increasing the risk of PMWS	Factors decreasing the risk of PMWS
Facilities	Large numbers of sows	Separate pit for adjacent fattening rooms
	Large pens at nursery and growing ages	Shower facilities
	Proximity to other pig farms	
Management practices	High level of cross-fostering	Sorting pigs by sex at nursery stage
	Short empty periods at weaning and fattening	Greater minimum weight at weaning
	Large range in age and weight entering to nursery	Group housing sows during pregnancy
	Continuous flow through nursery	Visitors avoiding contact with pigs for several days before visiting farm
	Purchase of replacement gilts	Use of semen from an insemination centre
	Sows with neck injuries due to poor injection technique	
	Early weaning (<21 days of age)	
Vaccination/ treatment/ nutrition	Vaccination of gilts against PRRSV	Vaccination of sows against atrophic rhinitis
	Vaccination of sows against *E. coli*	Regular treatment for ectoparasitism
	Use of separate vaccines against Erysipelas and parvovirus on gilts	Use of oxytocin during farrowing
		Use of spray-dried plasma in initial nursery ration

Taken from L. Grau-Roma et al. (2011) (6)

REFERENCES

REFERENCES

1. Isolation of porcine circovirus-like viruses from pigs with a wasting diseases in the USA and Europe. GM, Allan, et al. 1998, *J. Vet. Diagn. Invest.*, Vol. 10, pp. 3-10.

2. Porcine Circovirus Antigens in PK-15 Cell Line (ATCC CCL-33) and Evidence of Antibodies to Circovirus in Canadian Pigs. Dulac, Gilles C and Afshar, Ahmad. 1989, *Can. J. Vet Res*, Vol. 53, pp. 431-433.

3. Porcine circovirus type 2-associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. Opriessnig, Tanja, Meng, Xian-Jin and Halbur, Patrick G. 2007, *J. Vet. Diagn. Invest.*, Vol. 19, pp. 591-615.

4. Porcine circovirus: a minuscule yet mammoth paradox. Ramammorthy S, Meng XJ. 1, 2010, *Animal Health Research Reviews*, Vol. 10, págs. 1-20.

5. Porcine circoviruses: a review. Allan GM, Ellis JA. 2000, *J. Vet Diagn. Invest.*, Vol. 12, pp. 3-14.

6. Recent advances in the epidemiology, diagnosis and control of disease caused by porcine circovirus type 2. Grau-Roma L, Fraile L, Segales J. 1, 2011, *The Veterinary Journal*, Vol. 187, págs. 23-32.

7. Epidemiology of PCV2 and PCVAD. Rowland R, Henry S, Dritz S, Hesse R. San Diego, California : *Proc AASV*, 2008. pp. 469-470.

8. PCV2 studies: Research from the K-State PCV2 Team. Henry S, Tokach L, Dritz S, Nietfeld J, Rowland R, Hesse R. San Diego, California : *Proc AASV*, 2008. pp. 463-467.

9. Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case-control study supports a shift in genotypes with time. Dupont K, Nielsen EO, Baekbo P, Larsen LE. 2008, *Veterinary Microbiology*, Vol. 128, págs. 56-64.

10. Segales J, Olvera A, Grau-Roma L, Charreyre C, Nauwynck H, Larsen L, Dupont K, McCullough K, Ellis J, Krakowka S, Mankertz A, Fredholm M, Fossum C, Timmusk S, Stockhofe-Zurwieden N, Beattie V, Armstrong D, Grassland B, Baekbo P, Allan G. PCV-2 genotype definition and nomenclature. *Veterinary Record*. June de 2008. Vol. 162, 26, págs. 867-868.

11. Postweaning multisystemic wasting syndrome: a review of aetiology, diagnosis and pathology. Chae, C. 2004, The Veterinary Journal, Vol. 168, pp. 41-49.

12. A new method to identify cell types that support porcine circovirus type 2 replication in formalin-fixed, paraffin-embedded swine tissues. Perez-Martin E, Rovira A,

Calsamiglia, Mankertz A, Rodriguez F, Segales J. 2007, *Journal of Virological Methods*, Vol. 146, págs. 86-95.

13. A proposal on porcine circovirus type 2 (PCV2) genotype definition and their relation with postweaning multisystemic wasting syndrome (PMWS) ocurrence. Grau-Roma L, Crisci E, Sibila M, Lopez-Soria S, Nofrarias M, Cortey M, Fraile L, Olvera A, Segales J. 2008, *Veterinary Microbiology*, Vol. 128, págs. 23-35.

14. PCVD pathogenesis; consequences in vaccination against PCV2. Charreyre C, Andréoni C, Joisel F. San Diego, California : *Proc AASV*, 2008. pp. 165-172.

15. Seroprevalence of porcine circovirus type 2 in swine populations in Canada and Costa Rica. Liu Q, Wang L, Willson P, O'Connor B, Keenslide J, Chirino-Trejos M, Melendez R, Babiuk L. 2002, *Can J Vet Res*, Vol. 66, págs. 225-231.

16. The detection of porcine circovirus in the Australian pig herd. Raye W, Muhling J, Warfe L, Buddle JR, Palmer C, Wilcox GE. 5, May 2005, *Australian Veterinary Journal* , Vol. 83, pp. 300-304.

17. Comparative efficacy of three commercial PCV2 vaccines in conventionally reared pigs. Lyoo KS, Joo HS, Caldwell B, Kim HB, Davies PR, Torrison J. 2010, *The Veterinary Journal*, Vol. doi:10.1016/j.tvjl.2010.06.015.

18. Porcine circovirus type 2 (PCV2)- infection and re-inoculation with homologous or heterologous strains: virological, serological, pathological and clinical effects in growing pigs. Opriessnig T, Prickett JR, Madson DM, Shen HG, Juhan NM, Pogranichniy RM, Meng XJ, Halbur PG. 3, 2010, *Vet Res*, Vol. 41, pág. 31.

19. Comparision of efficacy of commercial one dose and two dose PCV2 vaccines using a mixed PRRSV-PCV2-SIV clinical infection model 2-3-months post vaccination. Opriessnig T, Patterson AR, Madson DM, Pal N, Halbur PG. 2009, *Vaccine,* Vol. 27, págs. 1002-1007.

20. Transient correlation between viremia levels and IL-10 expression in pigs subclinically infected with porcine circovirus type 2 (PCV2). Darwich L, Segalés J, Resendes A, Balasch M, Plana-Duran J, Mateu E. 2008, *Research in Veterinary Sciences*, Vol. 84, pp. 194-198.

21. Comparision of the effectiveness of passive (dam) versus active (piglet) immunization against porcine circovirus type 2 (PCV2) and impact of passively derived PCV2 vaccine-induced immunity on vaccination. Opriessnig T, Patterson AR, Madson DM, Pal N, Ramamoorthy S, Meng XJ, Halbur PG. 2010, *Veterinary Microbiology*, Vol. 142, págs. 177-183.

22. Pathological findings associated with naturally acquired porcine circovirus type 2 associated disease. Segales J, Rosell C, Domingo M. 2004, *Veterinary Microbiology*, Vol. 98, págs. 137-149.

23. Update on porcine circovirus and postweaning multisystemic wasting syndrome (PMWS). SD., Sorden. 3, 2000, *Swine Health and Production*, Vol. 8, págs. 133-136.

24. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). Harding, J. C.S. and Clark, E. G. 5, September and October 1997, *Swine Health and Production*, Vol. 5, pp. 201-203.

25. Postweaning multisystemic wasting syndrome: Epidemiology and clinical presentation. Harding, JCS, Clark EG, Strokappe JH, Willson PI, Ellis JA. 6, November and December 1998, *Swine Health and Production*, Vol. 6, pp. 249-254.

26. Clinical and pathological observations on pigs with postweaning multisystemic wasting syndrome. Quintana J, Segalés J, Rosell C, Calsamiglia M, Rodriguez-Arrioja GM, Chianini F, Folch JM, Maldonado J, Canal M, Plana-Duran J, Domingo M. 2001, *Veterinary Record*, Vol. 149, pp. 357-361.

27. Porcine circovirus diseases. Segales J, Allan GM, Domingo M. 2, 2005, *Animal Health Research Reviews*, Vol. 6, págs. 119-142.

28. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Fachinger V, Bischoff R, Jedidia SB, Saalmuller A, Elbers K. 2008, *Vaccine*, Vol. 26, págs. 1488-1499.

29. Detection rates of porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and swine influenza virus in porcine proliferative and necrotizing pneumonia. Drolet R, Larochelle R, Morin M, Delisle B, Magar R. 2003, *Vet Pathol*, Vol. 40, págs. 143-148.

30. Infectivity of porcine circovirus type 2 DNA in semen from experimentally-infected boars. Madson DM, RamammorthyS, Kuster C, Narinder P, Meng XJ, Halbur PG, Opriessnig. 2009, *Veterinary Research*, Vol. 40.

31. Lack of evidence of porcine circovirus type 1 and type 2 infection in piglets with congenital tremors in Korea. Ha Y, Jung K, Chae C. 2005, *Veterinary Record*, Vol. 156, págs. 383-384.

32. A cluster of farms experiencing severe porcine circovirus associated disease: Clinical features and association with the PCV2b genotype. Horlen KP, Schneider P, Anderson J, Nietfeld J, Henry S, Tokach LM, Rowland R. 5, 2007, *Journal of Swine Health and Production*, Vol. 15, pp. 270-278.

33. Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs with particular emphasis on the causative agent, the mode of transmission, the diagnostic tools and the control measures. A review. Ghebremariam, M. K. and Gruys, E. 3, 2005, *Veterinary Quarterly*, Vol. 27, pp. 105-116.

34. Derivation of porcine circovirus type 2-negative pigs from positive breeeding herds. Opriessnig, Tanja, et al. 4, 2004, *JSHAP*, Vol. 12.

35. PCR Detection and Evidence of Shedding of Porcine Circovirus Type 2 in Boar Semen. Larochelle R, Bielanski A, Muller P, Magar R. 12, 2000, *J. Clin. Microbiol.*, Vol. 38, pp. 4629-4632.

36. Pathogenesis of postweaning multisystemic wasting syndrome caused by Porcine circovirus 2: an immune riddle. Darwich L, Segales J, Mateu E. 2004, *Archives of Virology*, Vol. 149, págs. 857-874.

37. Porcine circovirus type 2: Current understanding of pathogenesis and role of coinfections. Opriessnig, T. Dallas, Texas : *Proc AASV*, 2009. págs. 491-497.

38. Puzzling over PCV2 immunology. Segalés J, Kekarainen T, Mateu E, Allan GM, Fossum C, McCullough K. Dallas, Texas : *Proc AASV*, 2009. pp. 487-490.

39. Silencing of natural interferon producing cell activation by porcine circovirus type 2 DNA. Vincent IE, Balmelli C, Meehan B, Allan G, Summerfield A, McCullough KC. 2007, *Immunology*, Vol. 120, págs. 47-56.

40. Immune responses and vaccine-induced immunity against Porcine circovirus type 2. Kekarainen T, McCullough K, Fort M, Fossum C, Segales J, Allan GM. 2010, *Veterinary Immunololgy and Immunopathology,* Vol. 136, págs. 185-193.

41. Activation of the Immune System is the Pivotal Event in the Production of Wasting Disease in Pigs Infected with Porcine Circovirus-2 (PCV-2). Krakowka S, Ellis JA, McNeilly F, Ringler S, Rings DM, Allan G. 2001, *Vet. Pathol*, Vol. 38, pp. 31-42.

42. Sow porcine circovirus type 2 (PCV2) status effect on litter mortality in postweaning multisystemic wasting syndrome (PMWS). Calsamiglia M, Fraile L, Espinal A, Cuxart A, Seminat C, Martin M, Mateu E, Domingo M, Segales J. 2007, *Research in Veterinary Science*, Vol. 82, págs. 299-304.

43. Individual risk factors for Post-weaning Multisystemic Wasting Syndrom (PMWS) in pigs: A hierarchial Bayesian survival analysis. Rose N, Eveno E, Grasland B, Nignol AC, Oger A, Jestin A, Madec F. 2009, *Preventive Veterinary Medicine*, Vol. 90, págs. 168-179.

44. Influence of maternal antibodies on efficacy of porcine circovirus type 2 (PCV2) vaccination to protect pigs from experimental infection with PCV2. Opriessnig T, Patterson AR, Elsener J, Meng XJ, Halbur PG. 3, 2008, *American Society for Microbiology* , Vol. 15, págs. 397-401.

45. Influence of husbandry and control measures on porcine circovirus type 2 (PCV-2) dynamics within a farrow-to-finish pig farm: A modelling approach. Andraud M, Rose N, Grasland B, Pierre JS, Jestin A, Madec F. 2009, *Preventive Veterinary Medicine*, Vol. 92, págs. 38-51.

46. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. Horlen KP, Dritz SS, Nietfeld JC, Henry SC, Hesse RA, Oberst R, Hays M, Anderson J, Rowland RR. 6, March 15, 2008, *JAVMA*, Vol. 232, pp. 906-912.

47. Experimental transmision of porcine circovirus type 2 (PCV2) in weaned pigs: a sequential study. Magar R, Larochelle R, Thibault S, Lamontagne L. 2000, *J. Comp. Path.*, Vol. 123, págs. 258-269.

48. Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). Pallarés FJ, Halbur PG, Opriessnig T, Sorden SD, Villar D, Janke BH, Yaeger MJ, Larson DJ, Schwartz KJ, Yoon KJ, Hoffman LJ. 2002, *J. Vet. Diagn. Invest.*, Vol. 14, pp. 515-519.

49. Effect of Vaccination with Selective Bacterins on Conventional Pigs Infetcted with Type 2 Porcine Circovirus. Opriessnig T, Yu S, Gallup J M, Evans RB, Fenaux M, Pallares F, Thacker EL, Brockus CW, Ackermann MR, Thomas P, Meng XJ, Halbur PG. 2003, *Vet. Pathol.*, Vol. 40, pp. 521-529.

50. Detection of neutralizing antibodies in postweaning multisystemic wasting syndrome (PMWS)-affected and non-PMWS-affected pigs. Fort M, Olvera A, Sibila M, Segales J, Mateu E. 2007, *Veterinary Microbiology*, Vol. 125, págs. 244-255.

51. Differences in virulence among porcine circovirus type 2 isolates are unrelated to cluster type 2a or 2b and prior infection provides heterologous protection. Opriessnig T, Ramamoorthy S, Madson DM, Patterson AR, Pal N, Carman S, Meng XJ, Halbur PG. 2008, *Journal of General Virology*, Vol. 89, págs. 2482-2491.

52. Snelson, Harry. AASV Board Approves Porcine Circovirus Position Statement. American Association Swine Veterinarians. [En línea] American Association Swine Veterinarians, 1 de November de 2006. [Citado el: 1 de February de 2011.] http://www.aasv.org/news/story.php?id=2131.

53. College Veterinary Medicine, Iowa State University. Detection of Anti-PCV2 antibodies. Iowa State University, College Veterinary Medicine. [En línea] 2010. [Citado el: Monday de February de 2011.] http://vetmed.iastate.edu/research/labs/pcv2/diagnosis-pcv2-associated-disease/available-tests/detection-anti-pcv2-anti.

54. An ORF2 protein-based ELISA for porcine circovirus type 2 antibodies in post-weaning multisystemic wasting syndrome. Blanchard Ph, Mahe D, Cariolet R, Truong C, Le Dimma M, Arnauld C, Rose N, Eveno E, Albina E, Madec F, Jestin A. 2003, *Vet Microbiol*, Vol. 94, págs. 183-194.

55. Modified indirect porcine circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV. Nawagitgul P, Harms PA, Morozov I, Thacker BJ, Sorden SD, Lekcharoensuk C, Paul PS. 1, 2002, *Clin Diagn Lab Immunol*, Vol. 9.

56. College Veterinary Medicine, Iowa State University. Detection of PCV2 Nucleic Acids. Iowa State University, College Veterinary Medicine. [En línea] 2010. [Citado el: 08 de February de 2011.] http://vetmed.iastate.edu/research/labs/pcv2/diagnosis-pcv2-associated-disease/available-tests/detection-pcv2-nucleic-a.

57. —. Detection of PCV2 Virus of Viral Antigen. Iowa State University, College Veterinary Medicine. [En línea] 2010. [Citado el: 08 de February de 2011.] http://vetmed.iastate.edu/research/labs/pcv2/diagnosis-pcv2-associated-disease/available-tests/detection-pcv2-virus-vir.

58. —. Further Characterization of PCV2 Isolate. Iowa State University, College Veterinary Medicine. [En línea] 2010. [Citado el: 08 de February de 2011.] http://vetmed.iastate.edu/research/labs/pcv2/diagnosis-pcv2-associated-disease/available-tests/further-characterization.

59. Virudical efficacy of nine commercial disinfectants against porcine circovirus type 2. Martin H, Le Potier MF, Maris P. 2008, *Veterinary Journal*, Vol. 177, págs. 388-393.

60. College Veterinary Medicine, Iowa State University. PCV2 vaccines. Iowa State University, College Veterinary Medicine. [En línea] 4 de May de 2010. [Citado el: 14 de February de 2011.] http://vetmed.iastate.edu/pcv2-vaccines.

61. Current knowledge and future research needed on PCV2 vaccines. Tanja, Opriessnig. St Paul : *Allen D. Leman Swine Conference*, 2008. págs. 63-67.

62. Emergence of a new type of porcine circovirus in swine (PCV): A type 1 and type 2 PCV recombinant. Gagnon CA, Music N, Fontaine G, Tremblay D, Harel J. 2010, *Veterinary Microbiology*, Vol. 144, págs. 18-23.

63. Field evaluation of the effects of a porcine circovirus type 2 vaccine on finishing pig growth performance, carcass characteristics, and mortality rate in a herd with a history of porcine circovirus-associated disease. Jacela JY, Dritz SS, DeRouchey JM, Tokach MD, Goodband RD, Nelssen JL. 1, 2011, *J Swine Health Prod*, Vol. 19, págs. 10-18.

64. Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. Kixmoller M, Ritzmann M, Eddicks M, Saalmuller A, Elbers K, Fachinger V. 2008, *Vaccine*, Vol. 26, págs. 3443-3451.

65. Biologic and economic benefits of controlling subclinical PCVAD with vaccination. King D, DuBois P, Painter T, Holck T, Edler R, Johnson C, Diaz E. San Diego, California : *Proc AASV*, 2008. págs. 159-161.

66. Effects of porcine circovirus type 2 (PCV2) vaccination on mortality and average daily gain. Feldamnn L, Cline G, Wilt V, Diaz E. San Diego, California : *Proc AASV*, 2008. págs. 317-319.

67. The Pig Disease Information Centre U.K. EU. Epidemic Wasting Diseases of Pigs. pighealth.com. [En línea] 2011. [Citado el: 15 de March de 2011.] www.pighealth.com/circovirus.htm.

68. Eikelenboom G, Walstra P, Huiskes JH. Species of meat animals: pigs. [aut. libro] Devine C, Dikeman M Jensen WK. Encyclopedia of Meat Sciences. Oxford : Elsevier, 2004, Vol. 3.

69. Kyriazakis I, Whittemore CT. Whittemore's science and practice of pig production. 3rd. s.l. : Blackwell Publishing, 2006.

70. Effect of antigenic challenge on growth and composition of agregated early-weaned pigs. Schinckel AP, Clark LK, Stevenson G, Knox KE, Nielsen J, Grant AL, Hancock DL, Turek. 1995, *Swine Health and Prod.*, Vol. 3, págs. 228-234.

71.Immunohistochemical detection of aetiological agents of proliferative and necrotizing pneumonia in italian pigs. Morandi F, Ostanello F, Fusaro L, Bacci B, Nigrelli A, Alborali L, Dottori M, Vezzoli F, Barigazzi G, Fiorentini L, Sala V, Leotti G, Joisel F, Sarli G. 2010, *Journal of Comparative Pathology.,* Vol. 142, pags. 74-78.

72. A field trial to evaluate a *Mycoplasma hyopneumoniae* vaccine: Effects on lung lesions and growth rates in swine. 1996, *Can Vet J.*, Vol. 37, pags. 299-302.

73. Pork Industry Handbook. Kauffman RG, Epley RJ, Carr RR. Extension bulletin (Michigan State University. Cooperative Extension Service); May, 1988, E-1222 (Revision).

74. The impact of PCV2 viremia in a high health canadian swine herd, a vaccination trial comparing two commercial vaccines. Reindl M, Dewey C, Vilaca K, de Grau F, Richardson K, Poljak Z. Saint Paul, Minnesota: *Proc. Allen D. Leman Swine Conference,* 2009. p. 170.

75. Effect of antigenic challenge in growth and composition of agregated early-weaned pigs. Schinkel AP, Clark LK, Stevenson G, Knox KE, Nielsen J, Grant AL, Hancock DL, Turek J.1995, *Swine Health and Prod.*, Vol. 3, pp. 228-234.

76. Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. Opriessnig T, Madson DM, Prickett JR, Kuhar D, Lunney JK, Elsener J, Halbur PG. 2008, *Veterinary Microbiology*, Vol. 131, pp. 103-114.

77. Experimental reproduction od postweaning mulstisystemic wasting syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and Porcine circovirus type 2. Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ, Halbur PG. 2004, *Vet. Pathol.*, Vol. 41, pp. 624-640.

78. Epidemiologic assessment of porcine circovirus type 2 coinfection with other pathogens in swine. Dorr PM, Baker RB, Almond G, Wayne S, Gebreyes WA. 2, 2007, *JAVMA*, Vol. 230, pp. 244-250.

79. Utilizing vaccination for porcine circovirus type 2 (PCV2) as a tool to aid elimination of PCV2 from swine populations. Megan, Potter. Mannhattan, Kansas: Kansas State University, 2011.

CHAPTER 2

Evaluating the use of sentinels to assess disease challenge from Porcine Circovirus type 2 in an endemically infected, vaccinated herd

María Cristina Venegas-Vargas, DVM; Barbara Straw, DVM, PhD.

MCVV: Department of Large Animal Clinical Science, College Veterinary Medicine, Michigan State University, Lansing, Michigan.

BS: 2554 CR 4740, Pomona, Missouri.

Corresponding author: Dr Maria Cristina Venegas-Vargas, D202 Vet Med Center, East Lansing, MI 48824; Fax: 517-432-1042; E-mail: venegasv@cvm.msu.edu

Support from David J. Ellis Swine Professorship

Abstract

Objective: To compare detection of viral circulation of Porcine circovirus type 2 (PCV2) and associated risk of clinical disease in a farm after implementation of PCV2 vaccine through the use of sentinels as compared to testing of vaccinates.

Procedures: Three replicates of 20 pigs each and one replicate of 22 pigs were weighed and ear tagged at weaning for use in the study. Pigs were matched in pairs by gender, weight and litter. Matched pairs of pigs were allocated to sentinel (non-vaccinated) and control (vaccinated) groups. Control pigs received a Killed Baculovirus vector PCV2 vaccine at weaning and 2 wks later per label directions. Blood samples were taken at weaning, 9 and 20 wks of age for Enzyme-linked immunosorbent assay

(ELISA), Indirect immunofluorescent assay 4 dilution (IFA-4 dilution) and PCV2 Polymerase chain reaction (PCR) (Pools of 2 animals of the same treatment group) testing.

Results: PCV2 DNA was detected in both sentinel (2 pools) and vaccinate (5 pools) populations. There was a significant difference in antibody titers at 9 and 20 wk of age ($P<0.0001$) with vaccinates having higher antibody titers. Average Daily Gain (ADG) and mortality were not statistically different between sentinel and control pigs ($p>0.05$).

Implications:

- Monitoring sentinels did not result in an increased detection of virus circulation in this study, regardless of which diagnostic test was used.
- The use of sentinel swine requires increased labor and time for detection, as well as presenting the potential for impaired animal wellness issues if clinical illness occurs.
- This study suggests that the use of PCV2 PCR on vaccinates to determine virus presence or absence is equivalent to the use of sentinels.

Keywords: swine, porcine circovirus, vaccine, herd health and production medicine

Porcine Circovirus type 2 is an important viral pathogen worldwide. Porcine circovirus associated disease (PCVAD) was first described in Canada in the early 1990s as Postweaning multisystemic wasting syndrome (PMWS) (1) (2) (3). PCV2 is excreted by affected pigs and clinically healthy pigs (4) (5). Not all pigs infected with PCV2 will develop disease; the outcome depends on the virus, host, timing of PCV2 infection, management related factors, coinfections and immune modulation (4) (6). In 2006, the first vaccine was available in USA. The benefits of vaccination are: improved post-weaning growth rate, decreased mortality rate, reduced viral load, increased ADG among finishing pigs and fewer lightweight pigs at marketing [A] (7) (8) (9) (10). Due to the clinical improvements seen with PCV2 vaccination, producers and veterinarians are starting to question if, once clinical symptoms have been reduced to a level of non-detection, it could be possible to end the vaccination without negative effects. There is the assumption that vaccination could minimize viral shedding and reduce environmental contamination to a no-effect level; allowing elimination of vaccination programs [A] (7) (8). In order to remove vaccination, it is necessary to evaluate virus shedding in swine herds. The Michigan State University (MSU) swine herd experienced moderately severe PMWS accompanied by 5-10% nursery mortality in 2006. A vaccination program was initiated in 2007. Signs of clinical disease disappeared after implementing the program and remained inapparent for the next 1.7 yrs. During this time, mortality of pigs in the nursery remained below 2%. Potter et al. (2009 personal communication) documented reduction in viral load (as measured by PCV2 PCR) in the MSU herd following vaccination. The purpose of this study was to compare the use of

sentinels vs. vaccinates for determination of viral load in vaccinated swine herds with no clinical signs of PCVAD

Materials and methods

This project was approved by the Michigan State University Institutional Animal Care and Use Committee.

Herd

The study was conducted at the Swine Teaching & Research Center at Michigan State University, a closed herd of 200 sows, located in East Lansing, Michigan. It represents a model of commercial, continuous-flow swine production. After a clinical outbreak of PCVAD, the farm started to use a PCV2 vaccine in May 2007. A study was performed on the farm before and after the implementation of the PCV2 vaccine. It revealed that prior to vaccination, maternally derived antibodies declined by 15 weeks of age in growing swine; with seroconversion following this decline and 76.2% of pooled serum PCR samples were PCV2 positive. After vaccination was initiated 100% of the pooled serum samples tested were PCV2 negative (using PCR) and there were no clinical symptoms nor necropsy diagnoses of PCVAD [A] (8). The farm is negative for Porcine Respiratory Reproductive Syndrome (PPRS), *Mycoplasma hyopneumoniae* and *Actinobacillus Pleuropneumoniae*. Cross-fostering of pigs is practiced.

Pigs

Eighty-two crossbred pigs were included in the study. Pigs were selected from litters of 35 sows arranged in 4 different batches of farrowing. This farm routinely farrowed 10-

15 sows every 4 weeks. The animals were born during the early winter period, i.e. from October until December 2008. Sow parity ranged from 1 to 8. On the day of weaning (approximately 3 weeks of age) the pigs were weighed and ear tagged. Pairs of matched pigs were created with respect to gender, weight and litter. One animal in each matched pair of pigs was assigned to non-vaccinated (sentinels) and vaccinated (control) groups respectively. There were 29 litters from which one matched pair of pigs was selected and 6 litters from which two sets of matched pigs were selected. All of the pigs from each farrowing group that were not included in the study underwent the farm's standard operating procedures which include vaccination for PCV2. After weaning the pigs were housed for approximately 6 weeks in nursery rooms in pens of 10 animals each. For the finishing phase, pigs were allocated in pens ranging in inventory from 10 to 15 pigs. The flooring was woven wire in the nursery section and concrete slatted in the finishing section. The study was concluded when the pigs reached 20 weeks of age.

PCV2 vaccine

The PCV2 vaccine administered in the study was a commercially available, killed, baculovirus-expressed, capsid protein-derived vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro Delaware). The vaccine was administered according to label instructions.

Study design

The study was designed as a controlled clinical trial. Three replicates of 20 pigs each and one replicate of 22 pigs were used. Each replicate corresponded to a sequential batch of farrowing sows. Pigs in the control group were given a 2 ml dose of the PCV2

vaccine intramuscularly in the neck at weaning (approximately 3 weeks of age). Two weeks later control group pigs received the booster dose of vaccine in accordance with the manufacturer´s recommendations. Pigs were weighed at weaning and at 20 weeks of age and overall average daily gain was calculated. In the nursery, trial pigs (both sentinels and vaccinates) were kept in separate pens in the same room, but with nose to nose contact with other pigs. Blood collection was performed at weaning (3 weeks of age prior to vaccination), 9 weeks of age and 20 weeks of age for all replicates but the first (The first replicate was not bled at 3 weeks of age). The serum from each pig was submitted to Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) for PCV2 PCR (The cut off to determine a sample as PCV2 positive was set at 40 Cycle threshold (Ct.)) (11), Open reading frame 2 (ORF2) ELISA and IFA-4 dilution tests. The samples were pooled (2 pigs/pool) for the PCR test. Pigs were pooled following numeric order within treatment group. If a pool was positive, the individual sera that made up the pool were not tested separately to determine if one or both were positive. Dead pigs were necropsied and a standard set of tissues submitted to a veterinary diagnostic laboratory at either Michigan State University or Iowa State University.

Statistical Analysis

For comparison of ELISA results between treatment groups the data were analyzed using the generalized linear mixed model. The replicates (4 groups) were considered a random effect. The pigs were considered the experimental unit. Treatment (non-vaccinated (sentinel) and vaccinated (control)) and time were included as fixed effects. Data for ADG were analyzed using linear mixed model. The replicates (4 groups) were

considered a random effect. The individual pig was used as the experimental unit. McNemar's exact test was used to investigate differences in mortality. Fisher´s exact test was used to analyze differences in PCR-PCV2 and the IFA 4-dilution. For all analyses, the significance level was set at $p<0.05$. Statistical analyses were performed using SAS software release 9.2 (SAS, Cary, NC: SAS Institute Inc.).

Results

PCR

There were 7 positive serum pools; 2 sentinel and 5 control. For the control group (vaccinates): two were positive at 3 weeks, two were positive at 9 weeks and one was positive at 20 weeks of age. For the sentinel group: one positive pool was detected at 3 weeks and one positive pool was at 9 weeks of age. There was not a significant difference in the proportion of positive pools between treatment groups (Table 3.2).

ELISA

There was not a significant difference in the levels of antibody (S:P) between treatment groups at weaning. There was a significant difference between treatments post weaning. The vaccinated group had higher levels of antibodies (Table 3.1) (Figure 3.2).

The interaction between treatment and time of sampling was statistically significant. The vaccinated group had higher antibody concentrations at 9 and 20 weeks of age compared to sentinels. Within the sentinel group, there was no difference in antibody concentrations post-weaning.

IFA-4 dilution

The level of antibodies against PCV2 was not statistically different between sentinels and vaccinated animals at weaning (P>.05). At 9 and 20 weeks of age the levels of antibodies against PCV2 were statistically different between sentinels and control pigs (P<0.001) (Figure 3.1).

ADG

The ADG between sentinel (721.2 grams) and vaccinated (703.1 grams) groups was not significantly different.

Mortality

The mortality rate was numerically higher but not statistically different between sentinel and vaccinated animals (7.32%, 4.88% respectively; P >0.05). None of the pigs developed clinical signs compatible with PCVAD during the trial. None of the pigs showed gross or histological lesions compatible with PCVAD.

Discussion

It has been suggested that the analysis of the serological profile for PCV2 may increase knowledge of viral circulation and may be useful for the implementation of vaccination strategies and effective control measures according to the characteristics of an individual herd (12). The objective of this study was to compare the use of sentinels as compared to vaccinates to detect PCV2 in a PCVAD symptom-free farm after almost 2 years of PCV2 vaccine implementation. In this study, there was no benefit to using sentinels for detection of circulating PCV2 virus as compared to testing of vaccinates. Both groups had PCV2 virus detected using PCR, and there was no difference between

the number of positive pools in each group. In fact, numerically, vaccinates had more positive pools than sentinel pigs, reinforcing that despite small sample size, there is no biological indication that sentinel are more likely to serve as a sensitive method of detection of circulating PCV2 in a vaccinated herd.

A proposed benefit of sentinel swine is the possibility of detecting increased antibody concentrations as a response to natural infection from circulating virus. Ramamoorthy and Meng (2010) reported that a typical response in PCV2 seropositive pigs in the field is characterized by a decrease in maternal antibody titers from 3 until 11 weeks of age, an increase in titers at 15 weeks and persisting PCV2 antibody titers thereafter (13). In this study, antibody detection methods were not helpful, as sentinel pigs did not demonstrate antibody responses consistent with natural PCV2 infection; despite being viremic (14) (15) (16). Vaccinate antibody responses were consistent with expected response to vaccine (10) (7). The decrease in antibody concentration from 3 weeks to 9 weeks in sentinels is in agreement with previous studies likely representing waning concentrations of maternal antibody (14) (16) (17) (15) (18). The results in the present study may be explained by exceptionally low levels of circulating virus. There is a reported lack of seroconversion in a Brazilian single farrow-to-finish farm that had not vaccinated sows or pigs against PCV2 that had history of PMWS 1.5 years prior to the investigation, but had no clinical signs during the study. The authors suggested the findings could be a result of low viral challenge throughout the productive cycle, which may have occurred as a result of sanitizing measures that probably decreased viral circulation and infection of the replacement animals (12). Although virus was detected

in this study, it would be expected that in a vaccinated herd, the viral load is exceptionally low, and there is pig to pig variability in antibody response to infection (14) (12).

A potential benefit of sentinels is detection of production impacts of sub-clinical infection through increased mortality or decreased production performance. There was no difference in pig performance between sentinels and vaccinates as measured by ADG and mortality in this study. Unlike several studies that reported reduction in mortality and ADG between vaccinated and non-vaccinated pigs, in the present study these parameters were not different between treatment groups, although has to be taken into consideration the absence of clinical PCVAD problems in the present farm (10) (19) (2) (7). The lack of association between vaccination status and performance may be explained by high level of herd immunity against PCV2, decreasing the circulating virus concentration to a level too low to result in production impairing disease. A limitation of our study is the power to detect a difference in ADG and mortality rate between these groups. Post-hoc power analysis indicated that we would have needed to see a difference of 58.97 g (0.13 lbs) in ADG with the sample size used, based on the difference found between treatment groups in a previous study [B].

Regarding Mortality, with the sample size used we would have needed to see a difference of twelve-fold or 29.3% in mortality rate between treatment groups.

One consideration is the determination of the appropriate ratio of sentinels to vaccinates pigs. It may be necessary to send more sentinel pigs through the system to be able to decrease the impact of herd immunity. To the best of our knowledge there

are no data regarding the number of immune animals necessary to protect a herd for PCV. Of course, increasing the number of animals susceptible to the virus could be very risky to the health status and performance of the farm. Increasing the number of sentinels to a proportion of the herd sufficient to detect circulating virus may be risk for an outbreak of PCVAD.

Another contributing factor may be the heath status of the herd included in this study. The high health status of the farm may contribute to the absence of clinical signs of PCVAD and low titers level of antibodies, due to the lack of coinfecting pathogens, that could trigger the immunosuppressive effect of PCV2 (2) (20) (21). Main (2009) reported that characteristic PCVAD lesions in tissue submissions coming from well-vaccinated pig populations were difficult to readily find (22); this is in accordance with the results in this study, where none of the tissues submitted to the lab showed any signs of PCVAD. Circovirus is ubiquitous, existing in essentially all swine herds; it cannot be diagnosed or ruled out based on mere presence or absence of the virus (10). The virus is highly resistant to conventional inactivation procedures making decontamination of premises difficult (9). Not all pigs infected with PCV2 will develop disease, the outcome depends on the virus, host, coinfections and immune modulation (4) (20). The immunological mechanism that causes one pig to have a subclinical presentation and another to be clinical is still unknown (20). Given this and the results of this trial, it can be concluded that PCV2 has not been eliminated from this farm. The current clinical decision is that

would be dangerous to stop the PCV2 vaccination program, and the farm maintains vaccination at the time of this submission.

Acknowledgements

Kevin Turner, BS; Wei Wang, PhD; Ron Bates, MS PhD; Julie Funk, DVM MS PhD from Michigan State University.

Erin Strait, PhD from Iowa State University

Footnotes

a. Megan Potter

b. Personal communication, In Press

APPENDICES

APPENDICES

Table 2.1

Least square mean (S/P ratios) ELISA, *P* value with same letter, are not significant different.

	Weaning	9 weeks	20 weeks
Sentinel	0.4499 [a]	0.1927 [b]	0.1067 [b]
Control	0.4602 [a]	0.7829 [c]	0.9630 [e]

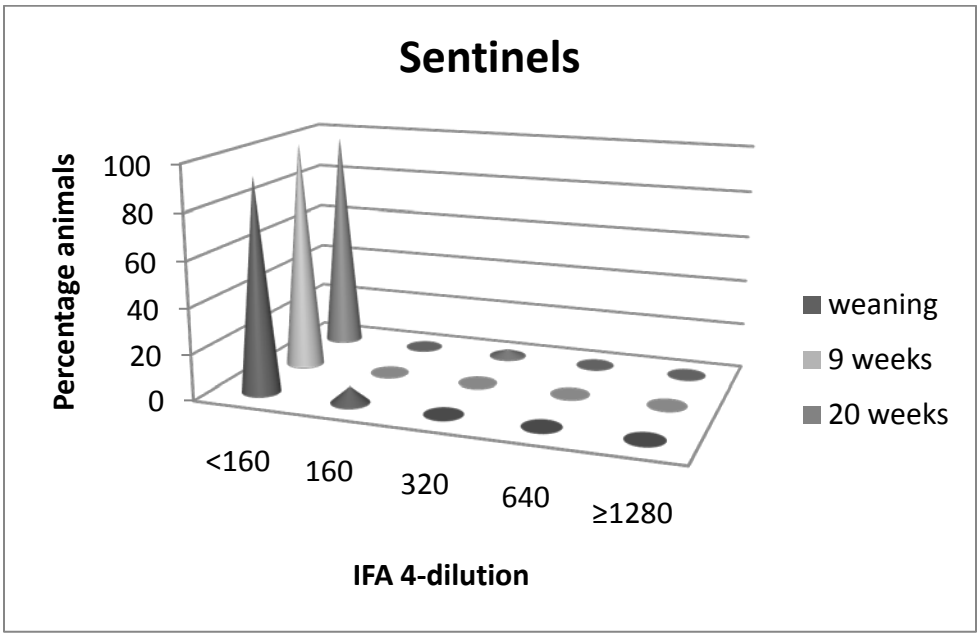
Table 2.2

Number of positive PCV2-PCR pools from the total PCV2-PCR pool samples.

Time of sampling	Sentinel	Control
Weaning	1 (15)	2 (14)
9 weeks	1 (19)	2 (19)
20 weeks	0 (18)	1 (19)

Figure 2.1

Distribution IFA 4-dilution titer levels in Sentinel and Control groups.



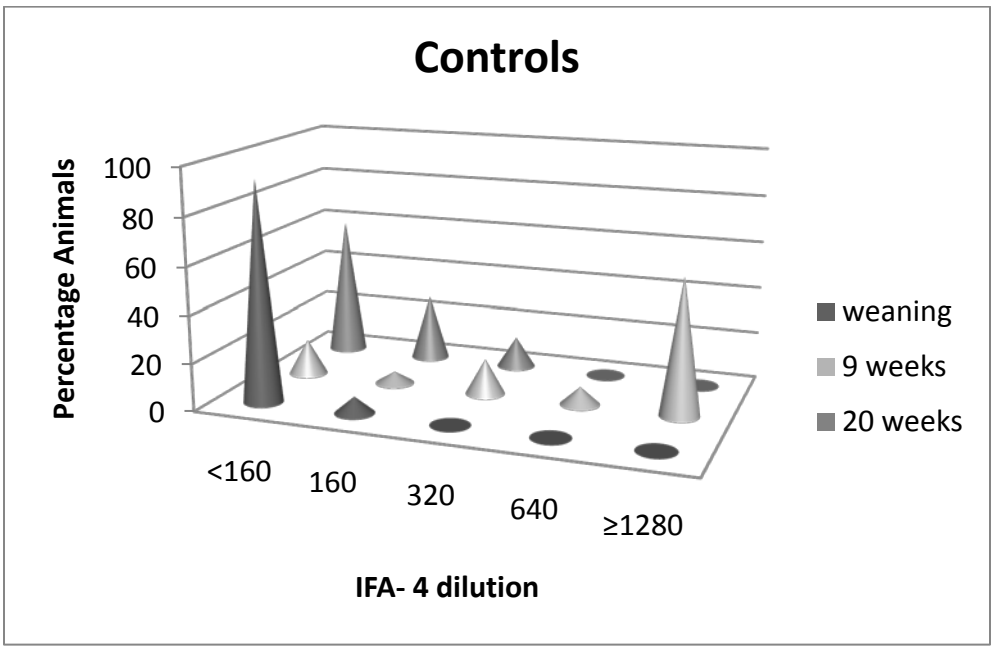


Figure 2.2

Distribution ELISA S/P ratios in Sentinel and Control groups

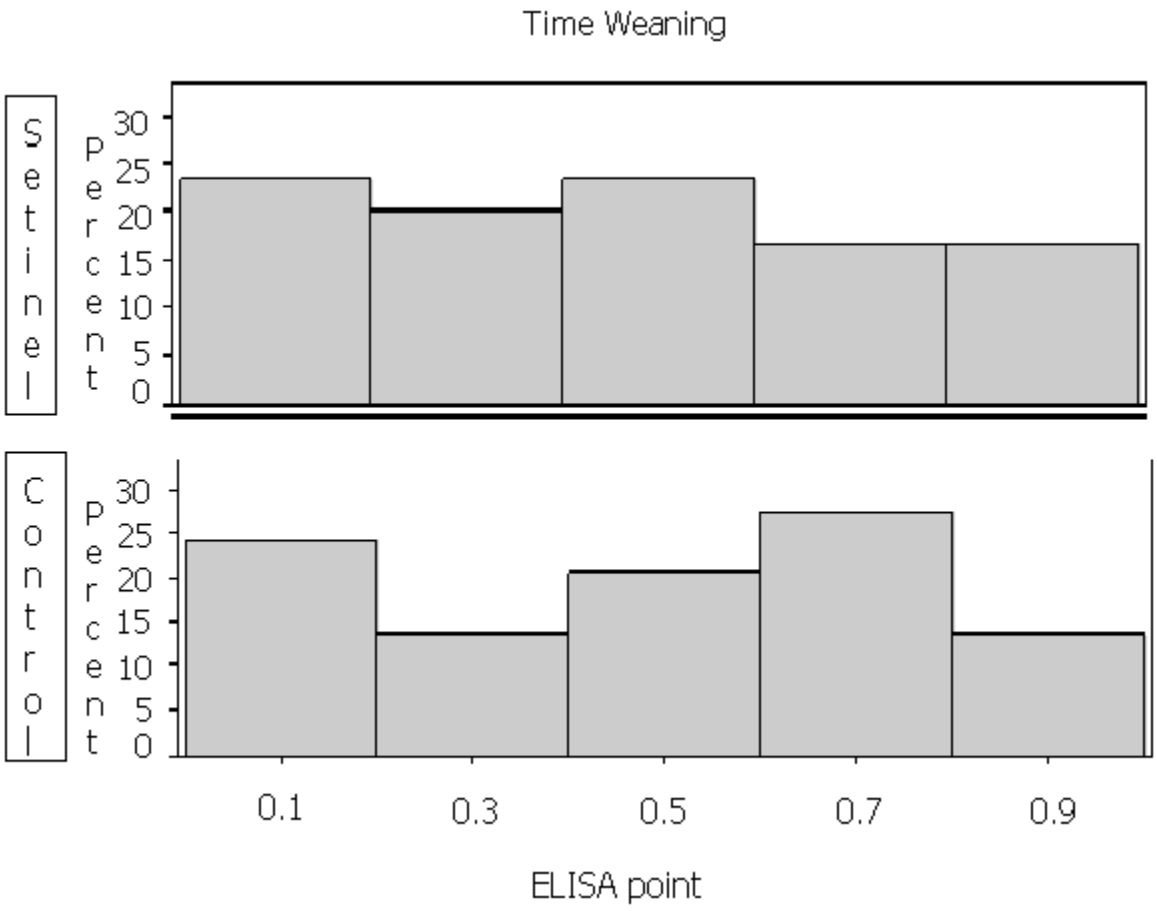


Figure 2.2 (cont'd)

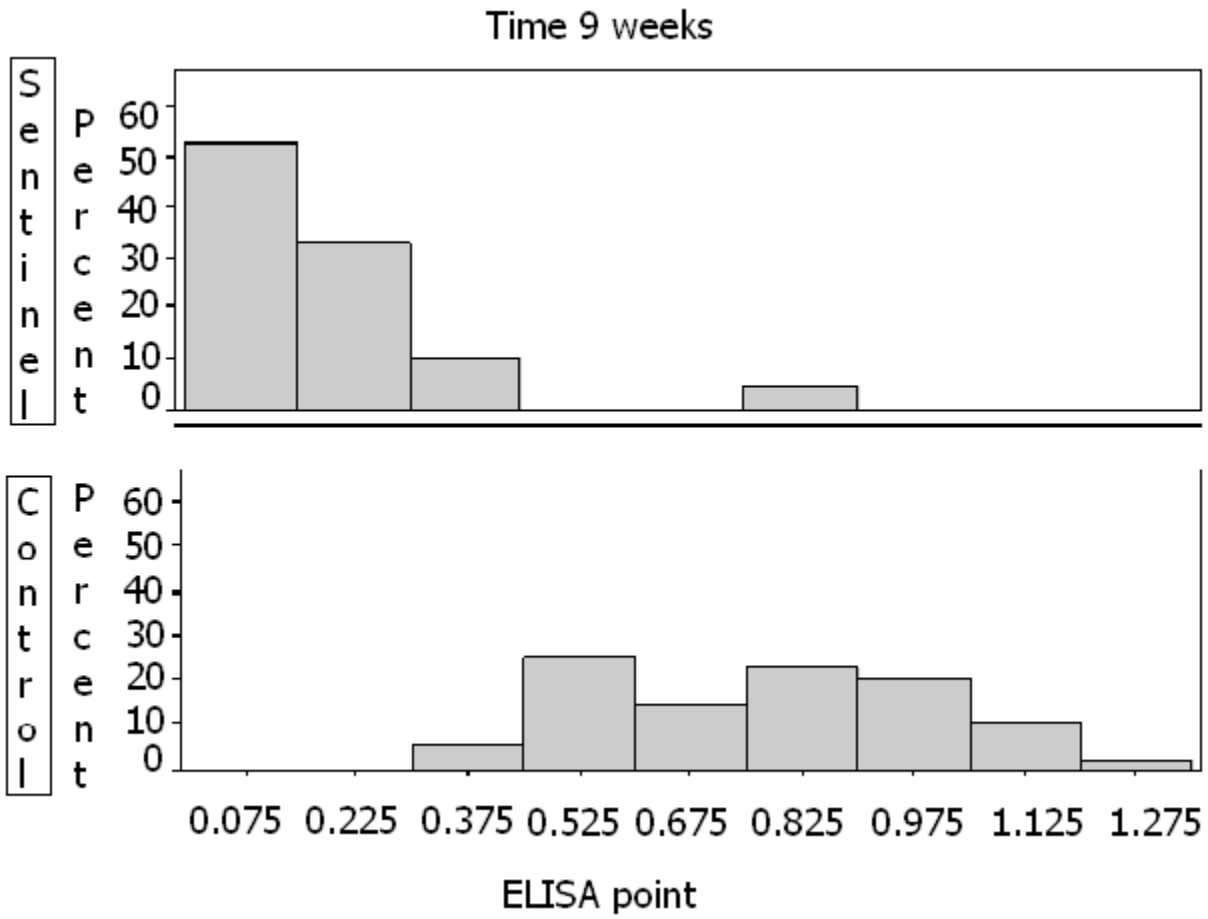
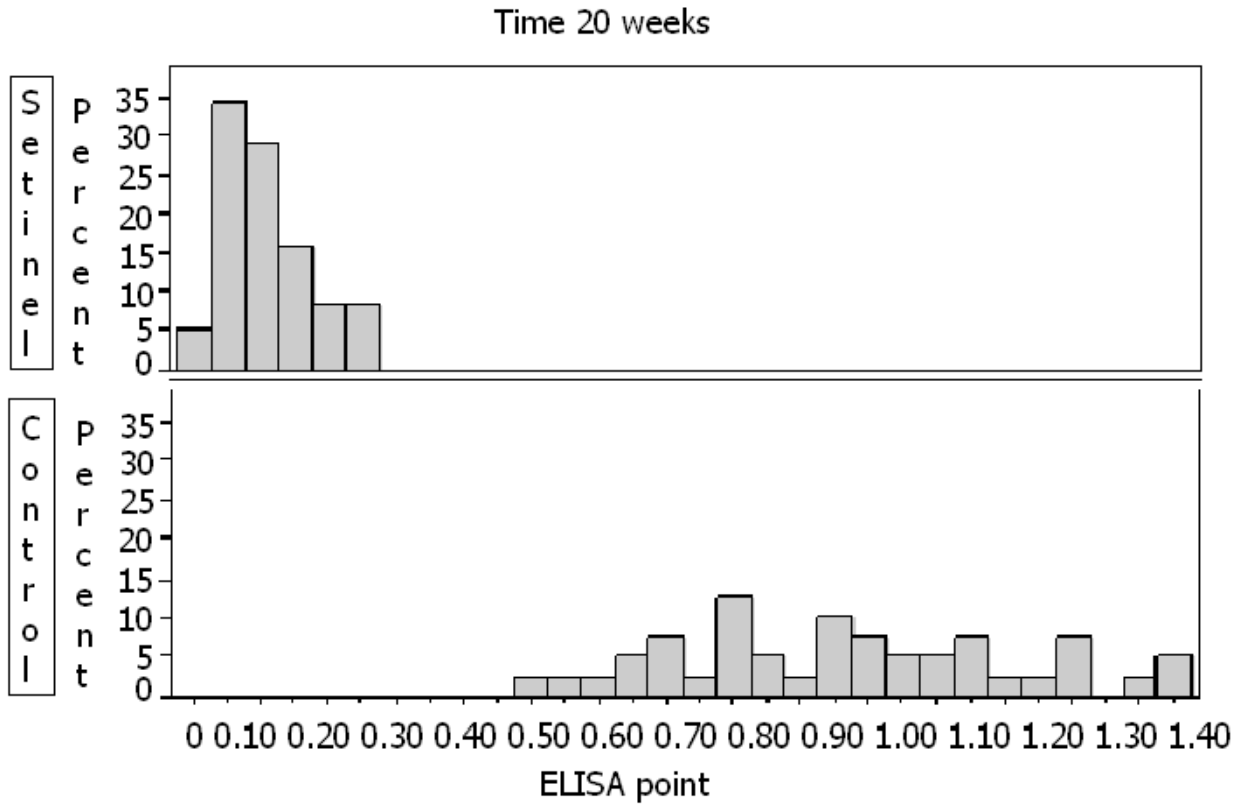


Figure 2.2 (cont'd)



REFERENCES

REFERENCES

1. Epidemiology of PCV2 and PCVAD. Rowland R, Henry S, Dritz S, Hesse R. San Diego, California : *Proc AASV*, 2008. pp. 469-470.

2. Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. Kixmoller M, Ritzmann M, Eddicks M, Saalmuller A, Elbers K, Fachinger V. 2008, *Vaccine*, Vol. 26, pp. 3443-3451.

3. Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. Magar, R., Muller, P. and Larochelle, R. 2000, *The Canadian Journal of Veterinary Research*, Vol. 64, pp. 184-186.

4. Porcine circovirus type 2-associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. Opriessnig, Tanja, Meng, Xian-Jin and Halbur, Patrick G. 2007, *J. Vet. Diagn. Invest.,* Vol. 19, pp. 591-615.

5. Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs with particular emphasis on the causative agent, the mode of transmission, the diagnostic tools and the control measures. A review. Ghebremariam, M. K. and Gruys, E. 3, 2005, *Veterinary Quarterly*, Vol. 27, pp. 105-116.

6. Recent advances in the epidemiology, diagnosis and control of disease caused by porcine circovirus type 2. Grau-Roma L, Fraile L, Segales J. 1, 2011, *The Veterinary Journal*, Vol. 187, pp. 23-32.

7. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. Horlen, K. P., et al. 6, March 15, 2008, *JAVMA*, Vol. 232, pp. 906-912.

8. Utilizing vaccination for porcine circovirus type 2 (PCV2) as a tool to aid elimination of PCV2 from swine populations. Megan, Potter. Manhattan, Kansas : Kansas State University, 2011.

9. Porcine circovirus type 2 (PCV2) vaccination of conventional pigs prevents viremia against PCV2 isolates of different genotypes and geographic origins. Fort M, Sibila M, Allepuz A, Mateu E, Roerink F, Segales J. 2008, *Vaccine*, Vol. 26, pp. 1063-1071.

10. PCV2 studies: Research from the K-State PCV2 Team. Henry S, Tokach L, Dritz S, Nietfeld J, Rowland R, Hesse R. San Diego, California : *Proc AASV*, 2008. pp. 463-467.

11. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 PCV. Opriessnig T, Yu S, Gallup JM, et al. 2003, *Journal of Veterinary Pathology*, Vol. 40, pp. 521-529.

12. Distribution of antibodies against porcine circovirus type-2 (PCV2) in single site and multi-site farrow-to-finish farms in Brazil. Gerber PF, Galinari GCF, Silva MX, Campos FS, Reis ACT. 2009, *Research in Veterunary Science*, Vol. 87, pp. 488-491.

13. Porcine circovirus: a minuscule yet mammoth paradox. Ramammorthy S, Meng XJ. 1, 2010, *Animal Health Research Reviews*, Vol. 10, pp. 1-20.

14. Detection on Porcine circovirus type 2 viremia and seroconversion in naturally infected pigs in a farrow-to-finish barn. McInstosh KA, Harding JCS, Ellis JA, Appleyard GD. 2006, *Canadial Journal Veterinary Research*, Vol. 70, pp. 58-61.

15. Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. Larochelle, Renee, Magar, Ronald and D' Allaire, Sylvie. 2003, *The Canadian Journal of Veterinary Research*, Vol. 67, pp. 114-120.

16. The levels of PCV2 specific antibodies and viremia in pigs. Carasova P, Celer V, Takacova K, Trundova M, Molinkova D, Lobova D, Smola J. 2007, *Research in Veterinary Sciences*, Vol. 83, pp. 274-278.

17. Derivation of porcine circovirus type 2-negative pigs from positive breeeding herds. Opriessnig, Tanja, et al. 4, July and August 2004, *Journal of Swine Health and Production*, Vol. 12.

18. Dynamics of porcine circovirus type 2 infection in a herd of pigs with postweaning multisystemic wasting syndrome. Rodriguez-Arrioja, Gabriela M. and al., et. 3, 2002, *American Journal Veterinary Research*, Vol. 63, pp. 354-357.

19. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Fachinger V, Bischoff R, Jedidia SB, et al. 2008, *Vaccine*, Vol. 26, pp. 1488-1499.

20. Puzzling over PCV2 immunology. Segales J, Kekarainen T, Mateu E, et al. Dallas, Texas : *Proceedings AASV,* 2009. pp. 487-490.

21. Porcine circovirus type 2: Current understanding of pathogenesis and role of coinfections. Tanja, Opriessnig. Dallas, Texas : *Proc AASV,* 2009. pp. 491-497.

22. Dealing with PCV2 vaccine failure in the field. Rodger, Main. Dallas, Texas : *Proc AASV*, 2009. pp. 505-506.

CHAPTER 3

Effect of porcine circovirus type 2 vaccine on postweaning performance and carcass composition

María Cristina Venegas-Vargas, DVM; Ronald Bates MS, PhD; Robert Morrison DVM, MBA, PhD; Dennis Villani, DVM, MBA; Barbara Straw, DVM, PhD.

MCVV: Department of Large Animal Clinical Science, College Veterinary Medicine, Michigan State University, Lansing, Michigan.

RB: Animal Sciences, Michigan State University, Lansing, Michigan.

RM: Swine Disease Eradication Center, College Veterinary Medicine, University of Minnesota, St Paul, Minnesota.

DV: Swine Veterinary Service, PC, Greensburg, Indiana.

BS: *2554 CR 4740, Pomona, Missouri.*

Corresponding author: Dr. Maria Cristina Venegas-Vargas, D202 Vet Med Center, East Lansing, MI 48824; Tel: 517-918-7372; Fax: 517-432-1042; E-mail: venegasv@cvm.msu.edu

Support from David J. Ellis Swine Professorship

Abstract

Objective: To evaluate the effect of porcine circovirus type 2 (PCV2) vaccination on average daily gain (ADG), mortality, carcass fat depth, loin depth, and percent lean.

Materials and methods: Pigs were weighed and ear-tagged 2 days prior to weaning to examine the influences of PCV2 vaccination on ADG, mortality, and carcass composition. Within litters, pigs were matched in pairs by gender and weight. Matched pairs of pigs were randomly allocated to Vaccinated and Control groups. Vaccinated pigs received a PCV2 killed baculovirus vector at weaning (approximately 3 weeks of age) and 3 weeks later. Pigs were weighed again at the end of the nursery phase and prior to marketing. Carcass data from the two groups of pigs were collected and compared.

Results: Vaccinated pigs had a significantly higher ADG than Control pigs. The overall ADG for Control pigs was 580.6 g per day, while Vaccinated pigs grew 630.5 g day ($P < .001$). More Vaccinated pigs (91%) went to primary markets than did control pigs (79%; $P < .01$). Vaccinated and Control pigs did not differ in carcass fat depth, loin depth, or percent lean ($P > .05$).

Implications: Under the conditions of this study, PCV2 vaccination has a large impact on growth rate and on the proportion of pigs going to primary markets, but not on carcass fat depth, loin depth, or percent lean, measurements that are used to determinate market value.

Keywords: swine, porcine circovirus, carcass composition, vaccine, herd health and production medicine

The effect of circovirus vaccination on mortality rate and performance has been reported in studies that compared vaccinated pigs with nonvaccinated pigs under field conditions. Some of the effects of porcine circovirus type 2 (PCV2) vaccination in pigs include greater ADG during the finishing period, lower finishing cull rates, and a lower probability of being lightweight at the time of marketing, compared to nonvaccinated pigs (1-3). Also, it has been reported that a greater percentage of vaccinated pigs than nonvaccinated pigs were marketed into the primary market chain (4). It is important to measure pig growth efficiency, but measures of efficiency should include the amount of lean edible pork produced and how desirable it is for processing and consumption (5). Carcass weight, fat depth, loin depth, and percent lean are the common carcass measurements implemented to establish the market value of pigs. These measurements also provide information to producers to improve carcass composition (6). If vaccination impacts carcass composition, this information is necessary to completely determine the cost-benefit ratio of employing vaccine. There are reports that in pigs vaccinated for PCV2 and *Mycoplasma hyopneumoniae,* carcass weight is greater than in nonvaccinated pigs (2) (7) (8) and that loin muscle depth is greater in PCV2-vaccinated pigs (7). But it was also reported that *M hyopneumoniae* vaccination had no effect on loin eye area, backfat thickness, or index, (8) and PCV2 vaccination had no effect on lean meat ratio (2). Disease suppresses appetite and therefore growth rate, which negatively impacts profitability. However, purposeful reduction in feed intake (limit feeding) has been used to reduce backfat of finishing pigs and improve carcass merit. Some diseases are catabolic and reduce muscle mass. Because of all the potential impacts of disease on

carcass composition, studies of vaccine effectiveness should include carcass measurements. The purpose of this study was to evaluate the effect of PCV2 vaccination on ADG, mortality, and carcass measurements.

Materials and methods

Study pigs were commercially owned animals managed under the standard operating procedures of the farm. Housing was standard within the industry for each phase of growth and the animals were humanely cared for. The National Pork Board's PQA Plus guidelines (www.pork.org) for animal care and handling were observed.

Herd

The study was conducted at an isolated 1200-sow farrow-to-wean farm in northeast Michigan without a history or clinical signs of porcine circovirus associated disease (PCVAD) and positive for *M hyopneumoniae* and porcine respiratory and reproductive syndrome (PRRS).The herd was originally composed of Landrace and Landrace × Large White sows. During the year before this study was initiated, replacement gilts and semen for artificial insemination were obtained from Newsham Choice Genetics, West Des Moines, Iowa. Mortality rates in the nursery (4%) and finisher (3.5%) were lower than those reported in herds which had experienced severe PCVAD (cumulative mortality rate 12.4% at finishing) (1). The farm had not previously used a PCV2 vaccine.

Pigs

Three hundred forty-eight unweaned crossbred pigs (19 days of age approximately), encompassing all 39 litters in one week's farrowing group, were included in the study. Parity distribution in that week's farrowing group was typical of the industry, with approximately 20% gilt litters and no sows over parity 8. Because maternal immunity was considered to have a major influence on disease status, treatment groups were determined as follows. At 2 days prior to weaning (approximately 3 weeks of age), all piglets within each litter were weighed on an electronic scale with an accuracy of 1%. Their weights were recorded on their backs, and the piglets were returned to the farrowing crate. Pigs were then assigned to pairs within a litter by weight and gender. Within a pair, vaccination with PCV2 vaccine (Vaccinated) or no treatment (Control) was determined by coin toss. Pigs were individually identified with ear tags at the time of treatment designation. After weaning, the pigs were housed for approximately 6 weeks in nursery rooms, stocked at 15 to 20 pigs per pen, with the two treatment groups mixed in the pens. Pigs were vaccinated against *M hyopneumoniae* during the fourth week in the nursery in accordance with the farm´s vaccination protocol. For the finishing phase, pigs were reallocated to 18 pens (2.4 m × 7.05 m), with the ratio of Control to Vaccinated pigs ranging from 3:7 to 7:3. The finishing barn (15 m × 240 m) was a curtain-sided building with a totally slatted floor. At 27 weeks of age, the first group of pigs was sent to slaughter, with the remaining pigs marketed in two batches at subsequent 2-week intervals. Pigs were loaded and penned in the truck by treatment designation and remained with their treatment groups through harvest. Carcass

measurements from 159 Vaccinated and 138 Control pigs were provided by the packing plant.

PCV2 vaccine

The PCV2 vaccine administered in the study was a commercially available killed baculovirus-expressed capsid-protein-derived vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, Delaware). The vaccine was administered according to label instructions.

Study design

The study was a controlled clinical trial. The pigs assigned to the Vaccinated group were given a single dose of the PCV2 vaccine (2 mL) intramuscularly in the neck 2 days before weaning (approximately 3 weeks of age). Three weeks later, pigs received the booster dose of vaccine in accordance with the manufacturer´s recommendations. All pigs were weighed before they were moved from nursery to the finishing buildings (8 weeks of age); 13 pens containing 186 of the study animals (87 Controls and 99 Vaccinated) were weighed at 22 weeks of age; and 11 pens containing 159 pigs (60 Controls and 99 Vaccinated) were weighed at 24 weeks of age. Normal management practices on the farm resulted in some pens containing non-trial pigs, so many pigs had to be moved from their pens to the scale to accomplish weighing. Two days were chosen to weigh pigs in order to have sufficient help to process them. Parameters calculated were average daily gain (ADG) for the nursery (weight gain ÷ the number of days from first PCV2 vaccination to the end of the nursery phase), ADG for finishing phase (weight gain ÷ the number of days from the end of nursery phase to 154 days

of age and 168 days of age), and overall ADG (weight gain ÷ the number of days from first PCV2 vaccination to 168 days of age). Pigs that were euthanized or died (without severe postmortem autolysis) were necropsied. When pigs were marketed, carcass weight, loin depth, fat depth, and lean percentage were obtained from the slaughter plant and compared for Vaccinates and Controls. Pigs were sent to slaughter in three loads at 2-week intervals, with each load composed of the pigs that had reached market weight at that time. In the slaughter plant, it was possible to track the treatment classification but not individual pig identification; treatment identification was possible through the ear tags and the location of the animals in different compartments in the truck. The first two loads were composed primarily of Vaccinated rather than Control pigs.

Statistical analysis

For carcass measurements, data were analyzed with a linear mixed model using the Mixed Procedure software in SAS version 9.1 (SAS Institute Inc, Cary, North Carolina). Slaughter day was considered a random effect. The treatment group within a load was the experimental unit. Treatment (Vaccinated versus Control) was included as a fixed effect. Carcass weight was adjusted to a common market age by including age at market as a covariate in the model. Data for ADG and live weight were analyzed with an ANOVA using General Linear Model software in SAS. The individual pig was the experimental unit. The model included the effects of treatment and gender. Results are reported as least squares means. Chi-square and Fisher´s exact test were used to

investigate possible differences in mortality between treatments. Values of $P < .05$ were considered significant.

Results

Live weight

At weaning and at the conclusion of the nursery phase, Vaccinated and Control pigs did not differ in weight (Table 1). However, at 22 and 24 weeks of age, Vaccinated pigs were significantly heavier than Control pigs.

Growth performance

There was no significant interaction between PCV2 vaccination and gender, demonstrating that the effect of vaccination was the same in males and females. From weaning until the end of nursery phase, ADG did not differ significantly between groups (Table 2.1). The ADG did differ between Control and Vaccinated groups from the end of nursery to 22 weeks of age and from the end of nursery to 24 weeks of age; overall ADG also differed between treatment groups (Table 2.1). Ninety-one percent of Vaccinated pigs and 79% of Control pigs went to primary markets ($P < .01$), these from the 174 pigs that initiated the study in each treatment group. A total of 162 Vaccinated pigs and 139 Control pigs were sent to the slaughter plant (primary market). It was possible to obtain the data form 159 Vaccinated pigs and 138 Control pigs from the slaughter. These data recovered at slaughter showed that the Vaccinated pigs (n = 159) produced 14,166.4 kg of carcass versus 12,077.4 kg from the Controls (n = 138). In live weight (data obtained from the killed sheet), the Vaccinated pigs were 3.16 kg

average heavier than the Controls and their value on average was $2.95 more per pig. This value is base on the price these pigs were market.

Carcass composition

In 159 Vaccinated pigs and 138 Controls at slaughter, carcass backfat depth (15.7 mm and 15.8 mm, respectively), loin depth (65.88 mm and 65.02 mm, respectively), and percent lean (57.09% and 56.91%, respectively) did not differ significantly between the two treatments (F test; $P > .05$).

Mortality rate

There was a numerical difference in the overall mortality rate between Controls (12 of 174; 6.9%) and Vaccinated pigs (5 of 174; 2.3%), but the difference was not significant ($P > .05$; relative risk [RR], 0.4; 95% confidence interval [CI], 0.13-1.08). Similarly, the numerical difference in mortality rate during the nursery phase was not statistically different between treatment groups: 2.4% for Vaccinated and 4.7% for Control ($P > .05$; RR 0.5; 95% CI, 0.14 to 1.65). In the finisher phase, mortality rate did not differ between Vaccinated and Control animals ($P > .05$; RR 0.22; 95% CI, 0.02-2.02). Of the 17 pigs that died during the study, 12 (eight Control and four Vaccinated) died during the nursery phase with no lesions characteristic of PCV2 infection, and five died in the finisher phase (four Controls and one Vaccinated) with mild macroscopic lesions of PCV2 infection. Histopathology was not performed at finishing.

Discussion

Results of this study indicate that in this herd, vaccination against PCV2 resulted in significantly greater ADG during the finishing phase and in fewer lightweight pigs at marketing.

The overall mortality rate, the mortality rate during the finishing phase, and the mortality rate during the nursery phase did not differ significantly between Vaccinated and Control groups. In this herd, PCV2 infection was subclinical, which can reduce growth rate but may have no detrimental effect on mortality, in accordance with previous studies (7) (9) (10). In subclinical cases, development of cellular immunity may limit the severity of disease expression (11)(12). The subclinical presentation of PCV2 in this herd was confirmed by the absence of lesions during the nursery phase and observation of mild macroscopic lesions during the finishing phase. These results do not agree with those of a previous study (13) that reported significant differences in mortality rate between vaccinated and nonvaccinated animals with subclinical PCVAD in a herd that was free of PRRS and *M hyopneumoniae*. However, these results are in agreement with two other studies (7) (10) that reported no difference in mortality rate between vaccinated and non-vaccinated pigs in herds with subclinical PCV2 infection: one of these herds was PRRS-negative and *M hyopneumoniae*-positive. During the nursery phase in the current study, PCV2 vaccination had no significant effect on ADG. From the end of nursery phase to 22 weeks of age, ADG was greater by 7.4% in Vaccinated pigs than in Control pigs. From the end of nursery phase to 24 weeks of age, ADG was greater by 9.4% in Vaccinated pigs than in Control pigs. Finally, ADG from weaning to 24 weeks was greater by 7.9% (49.9 g) in Vaccinated pigs than in

Controls. These results agree with those of other studies that evaluated the effects of PCV2 vaccination on growth performance (1- 3). In the current study, ADG was higher in Vaccinated pigs than in Controls, yet mortality rates in the two treatment groups were similar, suggesting that the herd experienced subclinical PCV2 infection, with mortality rate unaffected but with a negative effect on growth performance.

The immune response to disease-organism antigens is a major cause of reduced growth rate, as indicated by studies in several species (13) (14). In pigs, it has been reported to reduce live weight, growth, feed intake, and muscle growth during antigen challenge (14) Even though ADG in the current study differed significantly between Vaccinated and Control pigs, the common carcass measurements used to determine carcass value did not differ significantly between treatment groups. Carcass weight did not differ significantly between Control and Vaccinated pigs when adjusted to common market age. The major influences upon carcass yield are live weight, fatness, and genotype (15) Previous studies reported that pigs vaccinated against PCV2 had heavier carcass weights (2) (7). Vaccinated pigs grew faster and a greater percentage were marketed into primary markets; thus, under the conditions of this study (ie, vaccinating pigs in a herd subclinically infected with PCV2), vaccinated pigs can be marketed younger at a constant market weight, or will be heavier at a constant market age, than nonvaccinated pigs. This is in agreement with previous a study (4) that reported a higher percentage of vaccinated versus control pigs that were marketed into prime markets. In a study (8) that compared pigs vaccinated with a killed *Mycoplasma hyopneumoniae* vaccine with controls, carcass weight of vaccinated pigs was greater

and percentage of lightweight pigs was lower than the same parameters in nonvaccinated pigs, but there were no effects on other carcass characteristics. In a study that evaluated the effects of antigenic challenge (an *Escherichia coli* lipopolysaccharide and either modified-live or killed vaccines) on growth and composition of segregated early-weaned pigs, there was no significant difference in backfat depth measurements between control and antigen-treated pigs (14) In cattle, even though no clear mechanisms have been established linking disease and carcass traits, there is growing evidence that disease has the potential to affect not only carcass weight, but also the quantity, location, and ratio of muscle, fat, and water; more research is required to understand how cattle disease affects carcass traits (16). Exposure of animals to pathogenic or nonpathogenic antigens results in release of cytokines. In a previous study (17) it was demonstrated that administration of interleukin-1 and tumor necrosis factor induces anorexia, depresses protein synthesis, and stimulates protein degradation in skeletal muscle. Also, acute activation of the immune system via administration of nonpathogenic antigens results in lower voluntary feed intake, body growth rate, and efficiency of feed utilization in chicks and pigs (17). Minimizing the exposure of pigs to environmental antigens (pathogenic and nonpathogenic) consequently minimizes chronic activation of their immune systems, allowing them to express their whole potential for body growth, efficiency of food utilization, and carcass leanness (17).

In this study, backfat depth did not differ significantly between treatment groups. This herd's subclinical PCV2 status could explain why there was no difference in backfat

thickness between Vaccinated and Control pigs. Pigs clinically infected with PCV2 may have greater variation in weight gain, which would influence backfat depth (15). Since there was no significant difference between treatment groups for backfat and loin depth, consequently lean percentage also did not differ between Control and Vaccinated animals. This result does not agree with that in a previous study (7) that reported greater loin muscle depth in vaccinated pigs, but does agree with results of a study (2) that evaluated carcasses from pigs vaccinated against PCV2 and nonvaccinated controls. A Canadian field trial (13) reported that pigs that received one dose of PCV2 vaccine had fewer kilograms lean meat per carcass than pigs that received two doses of vaccine. In another study, (14) carcasses from control pigs had greater loin depth than carcasses from antigen-treated pigs. This trial controlled for carcass weight when backfat and loin eye were compared. In another trial (7) examining these traits, all pigs were marketed at a designated time, and body weight was considerably less in unvaccinated pigs.

Implications

- Under the conditions of this study, in a herd subclinically infected with PCV2, growth rate is better in pigs vaccinated for PCV2 than in nonvaccinated pigs.
- Pigs vaccinated for PCV2 may reach market weight faster than nonvaccinated pigs.
- PCV2 vaccination can be used to increase the proportion of pigs sold to the primary market.

- Under the conditions of this study, improvement in carcass fat depth, loin depth, and percent lean should not be anticipated after initiating a PCV2 vaccination program.

APPENDICES

APPENDICES

Table 3.1

Average weights (SD) of pigs in a commercial swine production facility, either vaccinated for PCV2 (Vaccinated) or not vaccinated (Control)*

Phase	Body weight (kg)				*P*†
	n	Vaccinated	n	Control	
Weaning‡	173	5.01 (0.09)	173	4.95 (0.09)	> .05
Nursery§	166	15.82 (0.27)	163	15.79 (0.27)	> .05
Finisher (22 weeks of age)	99	82.08 (1.12)	87	77.56 (1.22)	< .01
Finisher (24 weeks of age)	99	98.04 (1.42)	60	90.52 (2.05)	< .001

* Vaccinated pigs were vaccinated at 3 and 6 weeks of age with a killed PCV2 vaccine. Controls were neither vaccinated nor sham-vaccinated.

† ANOVA used to derive the *P* values.

‡ Pigs were weighed 2 days before they were weaned at approximately 21 days of age.

§ Pigs were weighed at 8 weeks of age before moving to the finisher.

PCV2 = porcine circovirus type 2

Table 3.2

Least squares means of average daily gain (g) in pigs in a commercial swine production facility, either vaccinated for PCV2 (Vaccinated) at 3 and 6 weeks of age or not vaccinated (Control)*

Phase	n	Vaccinated	SE	n	Control	SE	*P*†
Weaning to end of nursery	165	308.4	0.006	161	308.4	0.006	> .05
End of nursery to 22 weeks of age	99	671.3	0.01	86	621.4	0.01	.001
End of nursery to 24 weeks of age	98	725.7	0.02	57	657.7	0.01	.001
Weaning to 24 weeks of age	98	630.5	0.01	59	580.6	0.01	.001

* 174 pigs were assigned to each treatment group (Vaccinated and Control). Vaccinated animals received a killed PCV2 vaccine 2 days before weaning (approximately 3 weeks of age) and 3 weeks later. Pigs were weighed at approximately 3 weeks of age (346 pigs) and at the end of the nursery phase (8 weeks of age). Pigs were again weighed at either 22 or 24 weeks of age (variation due to the weighing process). Number of observations varied at each weigh date due to loss of pigs or the weighing process.

† F test used to derive the *P* values.

REFERENCES

REFERENCES

1. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. Horlen KP, Dritz SS, Nietfeld JC, Henry SC, Hesse RA, Oberst R, Hays M, Anderson J, Rowland RR. 2008, *JAVMA,* Vol. 232, pp. 906-912.

2. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Fachinger V, Bischoff R, Jedidia SB, Saalmüller A, Elbers K. 2008, *Vaccine,* Vol. 26, pp. 1488-1499.

3. Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. Kixmöller M, Ritzmann M, Eddicks M, Saalmüller A, Elbers K, Fachinger V. 2008, *Vaccine,* Vol. 26, pp. 3443-3451.

*4. Effects of porcine circovirus type 2 (PCV2) vaccination on mortality and average daily gain Feldmann L, Cline G, Wilt V, Diaz E. San Diego, California :*Proc AASV*, 2008. pp. 317-319.

5. Pork Industry Handbook. Extension bulletin (Michigan State University. Cooperative Extension Service). Kauffman RG, Epley RJ, Carr RR. May, 1988, E-1222 (Revision).

6. Eikelenboom G, Walstra P, Huiskes. Species of meat animals/pigs. In: Jensen WK, Carrick D, Dikeman M, eds. *Encyclopedia of Meat Sciences.* Vol 3. 1st ed. Oxford: Elsevier Ltd, 2004;1285- 1286.

*7. Biologic and economic benefits of controlling subclinical PCVAD with vaccination. King D, DuBois P, Painter T, Holck T, Edler R, Johnson C, Diaz E. San Diego, California : *Proc AASV*, 2008. pp. 159-161.

8. A field trial to evaluate a *Mycoplasma hyopneumoniae* vaccine: Effects on lung lesions and growth rates in swine. Dohoo IR, Montgomery ME. 1996, *Can Vet J,* Vol.37, pp. 299-302.

9. Porcine circovirus type 2-associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. Opriessnig T, Meng XJ, Halbur PG. 2007, *J Vet Diagn Invest,* Vol. 19, pp. 591-615.

*10. PCV2 studies: Research from the K-State PCV2 team. Henry S, Tokach L, Dritz S, Nietfeld J, Rowland R, Hesse R. San Diego, California : *Proc AASV*, 2008. pp. 463-467.

*11. PCVD pathogenesis; consequences in vaccination against PCV2. Charreyre C, Andréoni C, Joisel F. San Diego, California : *Proc AASV*, 2008. pp. 165-172.

12. Development of cell-mediated immunity to porcine circovirus type 2 (PCV2) in caesarean-derived, colostrums-deprived piglets. Fort M, Fernandez LT, Nofrarias M, Díaz I, Sibila M, Pujols J, Mateu E, Segalés J. 2009, *Vet Immunol Immunopathol*, Vol. 129, pp. 101-107.

*13. The impact of PCV2 viremia in a high health Canadian swine herd, a vaccination trial comparing two commercial vaccines. Reindl M, Dewey C, Vilaca K, de Grau F, Richardson K, Poljak Z. Saint Paul, Minnesota : *Proc Allen D. Leman Swine Conf*, 2009. pp. 170.

14. Effect of antigenic challenge on growth and composition of segregated early-weaned pigs. Schinckel AP, Clark LK, Stevenson G, Knox KE, Nielsen J, Grant AL, Hancock DL, Turek. 1995, *Swine Health Prod,* Vol. 3, pp. 228-234

15. Pig meat and carcass quality. Wood J, Whittemore C. In: Kyriazakis I, Whittemore CT. *Whittemore's Science and Practice of Pig Production*. 3rd ed. Oxford: Blackwell Publishing, 2006;46, 50-52.

16. Effect of cattle disease on carcass traits. Larson RL. 2005, *J Anim Sci,* Vol. 83(E Suppl):E37-E43.

17. Effect of chronic immune system activation on the rate, efficiency, and composition of growth and lysine needs of pigs fed from 6 to 27 kg. Williams NH, Stahly TS, Zimmerman DR. 1997, *J Anim Sci*, Vol. 75, pp. 2463-2471.

CHAPTER 4

Effect of PCV2 vaccine on carcass composition in a farm free of PRRS and *Mycoplasma hyopneumoniae*

María Cristina Venegas-Vargas, DVM; Ronald Bates MS, PhD; Robert Morrison DVM, MBA, PhD; Dennis Villani, DVM, MBA; Julie Funk, DVM, MS, PhD; Barbara Straw, DVM, PhD.

MCVV: Department of Large Animal Clinical Sciences, College Veterinary Medicine, Michigan State University, East Lansing, Michigan.

RB: Animal Sciences, Michigan State University, East Lansing, Michigan.

JF: Department of Large Animal Clinical Sciences and National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan.

RM: Swine Disease Eradication Center, College Veterinary Medicine, University of Minnesota, St Paul, Minnesota.

DV: Swine Veterinary Service, PC, Greensburg, Indiana.

BS: 2554 CR 4740, Pomona, Missouri.

Corresponding author: Dr. Maria Cristina Venegas-Vargas: D202 Vet Med Center, East Lansing, MI 48824; Fax: 517-432-1042; E-mail: venegasv@cvm.msu.edu

Support from David J. Ellis Swine Professorship

Abstract

Objective: To evaluate the effect of Porcine circovirus type 2 (PCV2) vaccination on Average Daily gain (ADG), mortality, primary market percentage, carcass fat depth, loin depth and percent lean in a commercial farm free of Porcine Respiratory Reproductive Syndrome (PRRS) and *Mycoplasma hyopneumoniae*.

Material and methods: The participating farm was located in north central Michigan. The farm was PRRS and *M. hyopneumoniae* negative. During the period of study the farm had subclinical Porcine circovirus associated disease (PCVAD). A total 200 barrows were used in the trial. Pigs were weighed and individually identified at two days prior to weaning. Assignment to treatment group was accomplished using pairs matched by weight and litter. One member of each matched pair was allocated to the vaccine and control groups respectively. Vaccinated pigs received a Killed Baculovirus vector PCV2 vaccine at weaning and three weeks later.

Results: There was a statistically significant difference in the proportion of pigs that went to primary market between vaccinates and controls (94% and 80% respectively). Vaccinated pigs had a significantly greater ADG in the finishing phase as compared to non-vaccinates, but there was no difference detected between treatment groups in measures of carcass composition and mortality.

Implications:

- PCV2 vaccination increases the proportion of pigs that reach the primary market.
- PCV2 vaccination did not have any effect on common carcass measurements.

- PCV2 may not improve the growth performance in a herd free of PRRS and *M. hyopneumoniae* as much as in a positive herd, but it still improves growth rate.

Circovirus vaccination has been reported to reduce mortality and improve performance (1) (2) (3) (4); even with subclinical presentations of PCV2 on farms (5). The immune response to pathogens is a major cause of the reduced growth rate as indicated by studies in several species (6) (7). Exposure of animals to pathogenic or nonpathogenic antigens results in the release of cytokines (8). In a previous study it was demonstrated that the administration of interleukin-1 and tumor necrosis factor induces anorexia, depresses protein synthesis and stimulates protein degradation in skeletal muscle (8). Co-infection with pathogens such as PRRS and *M. hyopneumoniae* is commonly described in pigs diagnosed with PCV2 infection (3) (9) (10) (11) (12) (13) (14). The association between the physiological effects of cytokine release in response to common pathogens would suggest there may be implications for carcass characteristics of swine infected with PCV2. Carcass weight, fat depth, loin depth and percent lean are common carcass measurements implemented to establish the market value of pigs (15). Another important economic factor for the producer is the quantity of the pigs that reach the primary market; because these pigs received the highest economic value. There is limited information on the consequence of vaccination for PCV2 on carcass composition and its repercussions for the cost effectiveness of using a vaccine. What little that has been published regarding the influence of PCV2 vaccination on carcass composition is contradictory. Some studies have reported improvements in carcass weight (5) (16) and loin muscle depth (5). But it had been also reported that vaccine does not have an effect on lean meat ratio (16), percentage lean, loin depth and backfat after adjusting to a common carcass weight (17). One potential

explanation for this difference is differences in health status of pigs receiving PCV2 vaccination. With this in mind, the objective of this study was to evaluate the effect of PCV2 vaccination on ADG, mortality rate, primary market percentage, carcass fat depth, loin depth and percent lean in a commercial farm that is negative for PRRS and *M. hyopneumoniae*.

Materials and methods

These were commercially owned animals managed under the standard operating procedures of the farm. The owner consent was obtained. Housing was standard within the industry for each phase of growth and the animals were humanely cared for.

Herd

The study was conducted in a multisite (farrow, nursery and finishing sites) farm in Northcentral Michigan. Clinical signs of PCVAD were not present at the time of the study. The herd was negative to *M. hyopneumoniae* and PRRS. The genetic make up of the pigs were Landrace x Large White.

Pigs

Two hundred crossbred barrows were enrolled in the trial. Two days prior to weaning (approximately 21 days of age) the pigs were weighed and individually identified using ear tags. Pairs of matched pigs were created with respect to weight and litter. One pig of the pair was assigned to the vaccinated group and the other pig of the pair was assigned to the control group. After weaning the pigs were housed in a nursery room for approximately 6 wks in pens ranging in inventory from 10 to 15 animals. Pigs were

moved at approximately 7.6 weeks of age to a finisher facility. The finishing barn was curtain-sided, total slatted building (6m x 6m pen dimension and 12m x 60m barn dimension). In the finishing barn, pigs were reallocated in 5 pens with a 1:1 ratio of control and vaccinated animals in each pen. At 21.7 weeks of age the first group of pigs was sent to slaughter, with the remaining pigs marketed in two batches in subsequent two weeks intervals. Pigs were loaded and penned in the truck by treatment designation and remained so through harvest.

PCV2 vaccine

The PCV2 vaccine administered in the study was a commercially available, killed, baculovirus-expressed, capsid protein-derived vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro Delaware). The vaccine was administered according to label instructions.

Study design

The study was a controlled clinical trial. Pigs in the vaccinated group were given a single dose of the PCV2 vaccine (2 ml) IM in the neck 2 days before weaning (approximately 3 weeks of age). Three weeks later pigs received the booster dose of vaccine in accordance with the manufacturer´s recommendations. Pigs were weighed before they were moved from the nursery to the finishing buildings (52 days of age). Before they were sent to slaughter the pigs were weighed again at 130 days of age. ADG for the nursery (weight gain divided by the number of days from vaccination to the end of nursery phase), ADG for finishing phase (weight gain divided by the number of days from the end of nursery to finishing weight day and overall ADG (weight gain

divided by the number of days from vaccination to finishing weight day) were calculated. Pigs that were euthanized or died without severe postmortem autolysis were necropsied. The percentage of pigs that reached the primary market was calculated. Primary market for this study was defined as pigs sold to the primary purchaser at full market weight. Non-primary market pigs were those animals classified as “light” and send to the local market plus those who died.

Average carcass measurements (loin depth, fat depth and lean percentage) of designated groups (92 Vaccinated and 82 Control pigs) were provided by the packing plant. Although treatment group designation was maintained during harvest, individual pig identification could not be associated with the individual carcass measurements. Pigs were sent to slaughter in three loads every two weeks. Each load was composed of the pigs that reached market weight at that time.

Statistical Analysis

The carcass measurements were analyzed with a linear mixed model using the Mixed procedure software in SAS version 9.1 (SAS Institute Inc, Cary, North Carolina). Slaughter day was considered a random effect. The treatment group (vaccinated or control) within a load was considered the experimental unit. Treatment was included as a fixed effect. Carcass weight was adjusted to a common market age by including age at market as a covariate in the model. Data for ADG and live weight were analyzed with an ANOVA using the General Linear Model software in SAS. The individual pig was used as the experiment unit. The model included the effects of treatment. Results are reported as least square means. Chi-Square and Fisher’s exact test were used to

investigate differences in mortality and proportion of pigs destined for primary market between treatment groups. Values of $P < 0.05$ were considered significant.

Results

Live weight

There was no significant difference in live weight between vaccinated and control pigs, at weaning (P= 0.90), nursery (P= 0.98), or finishing (P= 0.11) phases.

Growth Performance

There was no significant difference in the ADG in the nursery (P= 0.97). Conversely there was a significant difference in the ADG in finishing between treatment groups (P=0.04). For overall ADG, the difference was not statistically significant (P= 0.09) (Table 4.1).

Primary market

The percentage of pigs that went to primary market was greater in the vaccinated group compared with the control (94% vs. 80%, respectively (P= 0.005)).

Carcass composition

There was no difference in the fat depth (P= 0.28), loin depth (P= 0.94) and percent lean (P= 0.39) between vaccinated and control pigs. (Table 4.2).

Mortality Rate

There was only one pig mortality in the nursery phase (1 vaccinate). The overall mortality rate between vaccinated and control pigs was 2% and 5%, respectively (P=0.45).

Discussion

Decision-making regarding implementation of disease control measures can benefit from predictions of impact based on studies conducted in herds that are similar to the herd being considered for disease control (external validity). A factor that may impact the cost-benefit ratio of vaccine implementation is the disease burden of the herd. Previous research by our group and others (5) (16) (17) (18) has evaluated PCV2 vaccine performance in swine herds that have a health status that includes endemic PRRS and/or *M. hyopneumoniae* infection. Because of the well-described increased clinical severity demonstrated in pigs with co-infection (9) (19) (20) it would be expected that vaccine performance in herds without PRRS or *M. hyopneumoniae* may be less than that in herds with a greater disease burden.

The results of this study suggest that even in herds free of pathogens, considered endemic in the North American swine herd, vaccination for PCV2 improves growth performance and increases the proportion of swine harvested at primary market. The lack of evidence of improved ADG in the nursery phase alone is in concurrence with previous reports (5) (1) (16). The reported difference in ADG between vaccinated and control is less than reported in other studies (2) (6) (16) (17) and although disease burden may contribute to this difference, attribution of this as a causal explanation is beyond the scope of this research. Large-scale multiple farm-level clinical trials would be necessary to discern whether disease burden contributes to differences in growth performance benefits of PCV2 vaccination.

As it has been reported, chronic infection with pathogenic microorganisms causes negative metabolic effects and ultimately results in poor growth performance (17). It may be expected that animals vaccinated for PCV2 will have better growth performance -that could then be reflected in improved carcass composition parameters.

The results of this study indicated that even though the PCV2 vaccine had a positive impact in growth performance and primary marketing, the carcass characteristics were not different between the two treatment groups. This lack of effect of PCV2 immunization on carcass composition has been reported in others studies (18) (17) (16) with herds of varying health status. Yet other studies have reported a positive effect of PCV2 vaccination in one or more carcass measurements (5) (6). Based on this it can be proposed that the effect of PCV2 vaccine on carcass composition may vary farm to farm. The magnitude of the benefits from the vaccine will depend upon the interaction of multiple factors (9) (3) that form the equation for each individual farm. This is predictable as it is well known that PCV2 is a necessary but not a sufficient cause of PCVAD (4) (21). Farms can differ in factors like genetic composition, management practices and nutrition, all of them widely recognized as factors that influence the carcass characteristics, growth rate, live weight and mortality rate.

An important factor for interpretation and extrapolation of this data is the subclinical presentation that this farm had during the study period. The lack of clinical syndromes in this farm may explain the lack of difference in carcass characteristics between the treatment groups. Even though the mortality and the carcass measurements were not

significantly different between treatment groups, the higher percentage of pigs reaching primary market makes the implementation of PCV2 vaccination in high herd health status farm a plausible intervention on herds of high health status.

APPENDICES

APPENDICES

Table 4.1

Least square means for average daily gain and *P* values in PCV2 vaccinated and control pigs *.

Phase	Treatment						*P*
	n	Vaccinated	SE	n	Control	SE	
Weaning to end of nursery	100	335.6	7.53	99	335.6	7.57	0.97
End of nursery to 18.6 weeks of age	98	807.4	12.25	85	771.1	13.11	0.04
Weaning to 18.6 weeks of age	98	662.2	9.62	85	639.6	10.30	0.09

*100 pigs were assigned to each of the Vaccinated and Control treatment groups. Vaccinated animals received a PCV2 vaccine two days before weaning (approximately 3 weeks of age and 3 weeks later. Pigs were weighed at treatment designation and at the end of nursery phase (52 days of age). Pigs were again weighed at 18.6 weeks of age. Number of observation varied at each weigh date due to loss of pigs. The F test was used to derive the *P* values.

Table 4.2

Least square means of carcass measurements in PCV2 Vaccinated and Control pigs*.

	N	Backfat (mm)	Loin depth (mm)	Percent lean (%)
Vaccinated	92	26.16	59.18	51.76
Control	82	24.38	58.93	52.47

* Pigs were marketed in three loads, with each load was composed of pigs from each treatment group that reached market weight at that time. Group identity was maintained through harvest. Carcass weight was adjusted to a common market age. Carcass measurements were provided by the packing plant. None of the parameters differed between treatment groups (P > .05). The F test was used to derive the *P* values. Number of observations varied because of loss of pigs, and missing data from the slaughter plant.

REFERENCES

REFERENCES

1. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. Horlen KP, Dritz SS, Nietfeld JC, Henry SC, Hesse RA, Oberst R, Hays M, Anderson J, Rowland RR. 6, March 15, 2008, *JAVMA*, Vol. 232, pp. 906-912.

2. Effects of porcine circovirus type 2 (PCV2) vaccination on mortality and average daily gain. Feldamnn L, Cline G, Wilt V, Diaz E. San Diego, California : *Proc AASV*, 2008. pp. 317-319.

3. Recent advances in the epidemiology, diagnosis and control of disease caused by porcine circovirus type 2. Grau-Roma L, Fraile L, Segales J. 1, 2011, *The Veterinary Journal,* Vol. 187, pp. 23-32.

4. Immune responses and vaccine-induced immunity against Porcine circovirus type 2. Kekarainen T, McCullough K, Fort M, Fossum C, Segales J, Allan GM. 2010, *Veterinary Immunololgy and Immunopathology,* Vol. 136, pp. 185-193.

5. Biologic and economic benefits of controlling subclinical PCVAD with vaccination. King D, DuBois P, Painter T, Holck T, Edler R, Johnson C, Diaz E. San Diego, California : *Proc AASV*, 2008. pp. 159-161.

6. The impact of PCV2 viremia in a high health canadian swine herd, a vaccination trial comparing two commercial vaccines. Reindl M, Dewey C, Vilaca K, de Grau F, Richardson K, Poljak Z. Saint Paul, Minnesota : *Proc. Allen D. Leman Swine Conference*, 2009. p. 170.

7. Effect of antigenic challenge on growth and composition of agregated early-weaned pigs. Schinckel AP, Clark LK, Stevenson G, Knox KE, Nielsen J, Grant AL, Hancock DL, Turek. 1995, *Swine Health and Prod.,* Vol. 3, pp. 228-234.

8. Effect of chronic immune system activation on the rate, efficiency, and composition of growth and lysine needs of pigs fed from 6 to 27 kg. Williams, N. H., Stahly, T. S. and Zimmerman, D. R. 1997, *Journal Animal Science*, Vol. 75, pp. 2463-2471.

9. Porcine circovirus type 2-associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. Opriessnig, Tanja, Meng, Xian-Jin and Halbur, Patrick G. 2007, *J. Vet. Diagn. Invest.*, Vol. 19, pp. 591-615.

10. Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). Pallarés, F. J., et al. 2002, *J. Vet. Diagn. Invest.,* Vol. 14, pp. 515-519.

11. Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. Kixmoller, M. and al., et. 2008, *Vaccine*, Vol. 26, pp. 3443-3451.

12. Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. Opriessnig T, Madson DM, Prickett JR, Kuhar D, Lunney JK, Elsener J, Halbur PG. 2008, *Veterinary Microbiology*, Vol. 131, pp. 103-114.

13. Experimental reproduction od postweaning mulstisystemic wasting syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and Porcine circovirus type 2. Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ, Halbur PG. 2004, *Vet. Pathol.*, Vol. 41, pp. 624-640.

14. Epidemiologic assessment of porcine circovirus type 2 coinfection with other pathogens in swine. Dorr PM, Baker RB, Almond G, Wayne S, Gebreyes WA. 2, 2007, *JAVMA*, Vol. 230, pp. 244-250.

15. Eikelenboom G, Walstra P, Huiskes. Species of meat animals/pigs. [book auth.] Carrick D, Dikeman M Jensen WK. Encyclopedia of Meat Sciences. 1st. Oxford : Elsevier Ltd., 2004, Vol. 3, pp. 1285-1286.

16. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Fachinger V, Bischoff R, Jedidia SB, et al. 2008, *Vaccine*, Vol. 26, pp. 1488-1499.

17. Field evaluation of the effects of a porcine circovirus type 2 vaccine on finishing pig growth performance, carcass characteristics, and mortality rate in a herd with a history

of porcine circovirus-associated disease. Jacela JY, Dritz SS, DeRouchey JM, Tokach MD, Goodband RD, Nelssen JL. 1, 2011, *J Swine Health Prod*, Vol. 19, pp. 10-18.

18. Effect of porcine circovirus type 2 vaccine on postweaning performance and carcass composition. Venegas-Vargas MC, Bates R, Morrison R, Villani D, Straw B. In Press, JSHAP.

19. Porcine circovirus type 2: Current understanding of pathogenesis and role of coinfections. Opriessnig, Tanja. Dallas, Texas : *Proc AASV*, 2009. pp. 491-497.

20. Porcine circovirus: a minuscule yet mammoth paradox. Ramammorthy S, Meng XJ. 1, 2010, *Animal Health Research Reviews*, Vol. 10, pp. 1-20.

21. Pathological findings associated with naturally acquired porcine circovirus type 2 associated disease. Segales J, Rosell C, Domingo M. 2004, *Veterinary Microbiology*, Vol. 98, pp. 137-149.

Summary

Veterinarians and producers face many challenges in management of PCVAD on swine farms. Among these challenges is evaluating the efficacy of vaccination programs. One challenge is determining whether virus is still circulating on vaccinated farms without clinical disease symptoms. In these situations the concentration of circulating virus is low, and increasing the probability that diagnostic test may not be able to detect this low viral load. One potential solution is the use of non-vaccinated sentinel animals to detect the virus. The use of sentinels requires increased time and effort, and at least in this study, did not result in greater sensitivity for detections of virus circulating in pigs as compared to standard diagnostic test applied to vaccinated pigs. A second challenge for producers is to determine whether vaccination is efficacious in herds with subclinical disease. The challenge of this determination is that, due to PCVAD clinical presentation and severity is dependant upon not only PCV2 infection, but also is effected by concurrent infections with other pathogens, as well as management factors. Therefore, the impact of PCV2 vaccination will likely vary by herd dependent upon these factors. In this study, in 2 herds with PCV2 subclinical presentation, but different endemic disease burdens based on PRRS and M. hyopneumoniae status, vaccinated pigs had improved growth rate and a higher proportion of animals reaching primary market compared to non-vaccinated pigs. Furthermore, there was no difference on carcass backfat depth, loin depth and percentage lean between PCV2 vaccinated and non-vaccinated animals in either herd.

These results provide veterinarians and pork producers important information on both monitoring viral circulation on vaccinated swine herds, and data for determining the benefits of PCV2 vaccination on commercial swine herds. Future research to further evaluate the determination of a herd classification scheme that would allow the termination of PCV2 vaccination, as well as evaluation of carcass characteristics in vaccinated and non-vaccinated pig, are needed.